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CONTENTS

PAGE

Experiments on Local Specific Therapy in Poliomyelitis. The Utilization of Hypertonic Solutions in the Serum Treatment of Experimental Poliomyelitis.
By W. L. Aycock, M. D. and H. L. Amoss, M. D. . . 361

Clasmatocytes and Tumor Cells in Cultures of Mouse Sarcoma. (Illustrated)
By Warren H. Lewis and George O. Gey 369

A Note on the Effect of the Intravenous Administration of Mercurochrome on the Bacteriostatic Action of Blood.
By J. H. Hill and J. A. C. Colston 372

Reversible Gelation in Living Cells.
By Margaret Reed Lewis 373

Observations on Strains of Hemolytic Streptococci Recovered from Carriers and Cases During an Outbreak of Acute Tonsillitis.
By Augustus R. Felty and Arthur L. Bloomfield 379

PAGE

Precipitin Reactions of a Crystalline Globulin from Human Urine.
By H. S. Everett, S. Bayne-Jones, and D. Wright Wilson 385

The Rate of Locomotion of Ameba in Alkali Chlorides.
By J. Graham Edwards and Harrison S. Forgrave, Jr. 387

Poikilocytes in Otherwise Normal Blood. (Elliptical Human Erythrocytes.) (Illustrated)
By John G. Huck, M. D. and Rena M. Bigalow, M. D. 390

On the Mode of Spread of an Outbreak of Acute Tonsillitis.
By Arthur L. Bloomfield and Augustus R. Felty . 393

Notes on New Books 398

Books Received 399

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EXPERIMENTS ON LOCAL SPECIFIC THERAPY IN POLIOMYELITIS
THE UTILIZATION OF HYPERTONIC SOLUTIONS IN THE SERUM TREATMENT OF EXPERIMENTAL POLIOMYELITIS

By W. L. Aycock, M. D. and H. L. Amoss, M. D.

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The blood sera of human beings who have recovered from an attack of poliomyelitis and of monkeys *(Macacus rhesus)* which have recovered from the experimental disease are known to contain antibodies active against the virus,[2,7,16] but the production of an artificial immune serum (hyper-immune) has not yet been accomplished. Favorable results have been obtained by the use of blood serum from human convalescents in the treatment of the experimental infection in monkeys [17,18] and of the disease in human beings.[15,19,20,21] However, these results have fallen short of the expectations which the demonstrated immunological properties of this serum would seem to warrant. The methods heretofore employed in the serum treatment of this disease have been chiefly intravenous and intraspinous injections, but a consideration of the modern conception of the circulation of the fluids of the central nervous system tissues raises the question as to whether or not these methods of administration provide the optimal conditions for the therapeutic action of the serum. Since human immune serum can be obtained only in limited quantity and on account of the fact that it cannot be expected, by reason of its low potency, to yield the results which might be obtained with an hyper-immune serum, it is important that, pending the development of a more efficacious serum, every effort be made to obtain the maximal effect of this serum.

* Maintained by a special fund privately donated.

Recent advances in the knowledge of the circulation of fluids of the central nervous system and the experimental production of alterations of these fluid currents suggest possibilities in this connection, and form the basis of the experiments to be recorded here.

Mechanism of Local Specific Therapy

Effective local specific therapy in diseases of parasitic origin may be accomplished where it is possible to cause a suitable chemical or biologic agent to act on the parasites in a sufficient degree of concentration and for such a period of time as is necessary to accomplish their destruction without, of course, causing undue injury to the tissues of the host.[32,37] It has been shown, for example, that the higher the concentration of the specific curative agent about the parasites in trypanosomal, meningococcal, and other infections, the greater is the assurance of their complete destruction. The availability of any remedial agent introduced into the body for this purpose is determined by its distribution there, and this in turn is dependent upon (a) the ability of a substance when introduced into one portion of the body to be carried by its physical or chemical affinities to other portions which harbor the micro-organisms, or (b) the ability to introduce the substance directly into the focus of the infection. There are excellent instances of the fortunate combination of circumstances which permit the utilization of each of these mechanisms for obtaining the desired distribution of remedial agents. For example, tetanus antitoxin introduced into the subcutis for prophylactic purposes is carried to other points at which its action is desired in sufficient amount to bring about the neutralization of the toxin as it is liberated at the seat of the infection; whereas the fact that one is able to introduce antimeningococcic serum directly into the subarachnoid space makes possible a therapy of a degree of effectiveness which could not be brought to bear upon this disease if it were possible to introduce the serum into the circulation only, for example, and to rely upon its affinities for tissues to reach the lesions within the meninges.

In other instances the circumstances are not so fortunate. By reason of the fact that the parasites are so situated within the body that they do not lie within the paths of distribution of the particular therapeutic agent which are the result of its affinities for certain tissues, or if the parasites lie in a region of the body into which the curative agent may not be introduced directly, then either of the mechanisms referred to becomes ineffective. This is especially true of the tissues of the central nervous system. It is a striking fact that therapy in diseases in which the parasites exist within the central nervous system tissue is lamentably ineffective, despite the fact that the chemicals or sera employed, as evidenced by tests against the micro-organisms concerned, are suitable for their complete destruction. That this failure, at least in some instances, is due to the inability of the medication to reach the parasites is amply shown by the fact that lesions in more accessible portions of the body caused by the same organisms respond readily to such remedies, as, for example, in the case of syphilis of other tissues as compared with syphilis of the central nervous system.

The virus of poliomyelitis has not been found in the blood or cerebrospinal fluid at any stage of the disease in human beings.[6,36] In certain experiments on the route of infection, in which the monkey was used as a test animal, the virus was detected in the cerebrospinal fluid soon after its injection intravenously and in other experiments after its application to the nasal mucosa. However, after intravenous injection it tends to disappear from the blood stream so that after 120 hours it can no longer be detected there,[35] and although the virus was present in the cerebrospinal fluid on the third day after intracerebral inoculation,[9] it was not demonstrable in it after the onset of symptoms of the experimental disease in the monkey.[4] Thus, any appearance of the virus in the blood or in the cerebrospinal fluid is transitory and may be regarded as representing a stage in its passage from the exterior to the central nervous system tissue. Once localized in the central nervous system tissue, it does not again appear in the blood or cerebrospinal fluid during the course of the infection. Hence it would appear that the introduction of immune serum into either one of these fluids does not of itself insure contact of the immune serum with the invading virus.

Intravenous Serum Treatment

Any substance in order to be delivered to the tissue cells from the circulation must pass through the walls of the capillaries. This endothelial wall exercises a high degree of specificity in regard to the substances which it allows to pass. This discriminatory power of the capillaries varies in different portions of the body in accordance with the needs of the particular tissues concerned, and in the case of the capillaries of the central nervous system, namely, the vessels of the brain and spinal cord and the specialized choroid plexus, it reaches a high degree of perfection.[33] These vessels are known to be capable of excluding most substances, including immunity principles, which may be present in the blood, from the tissue of the central nervous system.[34] The cerebrospinal fluid is derived from the blood stream through the medium of these capillary systems and the character of this fluid itself is evidence of the precision of the apparatus which secretes it. These considerations indicate that immune serum introduced into the circulation would be entirely excluded from the tissue spaces of the central nervous system and this seems to be the case, provided the capillary walls which guard the entrance to these tissue spaces are intact. However, certain changes occur, or

may be induced, which alter the permeability of these vessels, and these should be taken into consideration if a proper understanding of the action of intravenous injection upon the central nervous system is to be arrived at.

Flexner and Amoss [8] found that an irritation or even a slight alteration in the integrity of the meninges or choroid plexus, resulting from the subarachnoid injection of normal horse serum or other substances, permits the virus of poliomyelitis, when introduced into the circulation, to pass to the central nervous tissue and set up infection. Moreover, they demonstrated that immunity principles, although present in the blood as early as the sixth day of the disease,[17] pass to the cerebrospinal fluid with difficulty,[3] and suggest that the inflammatory condition present in the meninges might account for this passage of immunity principles from the blood to the cerebrospinal fluid and thus comprise a fortunate element in the pathological events of the disease. Flexner and Amoss have also shown that immunity principles, when injected into the blood stream, can be made to pass to the cerebrospinal fluid under conditions in which the meninges have been experimentally inflamed.[3] Numerous observations of a similar nature have been recorded [27,28,29,30] in respect to the passage of other substances from the circulation to the cerebrospinal fluid, and form the basis of the clinical application of an artificial disturbance of the relation between the blood stream and the cerebrospinal fluid spaces in the treatment of certain diseases of the central nervous system.

The term "permeability of the meninges" has come into rather common use in reference to the passage of substances from the blood stream to the cerebrospinal fluid, but the exact manner in which such passage takes place is not yet known.

The fluid of the subarachnoid spaces is the combined product of the choroid plexus and the capillaries within the central nervous tissue itself.[31] These structures, as well as the capillaries of the meninges and the posterior root ganglia,[22] have been suggested as possible points of entrance into the central nervous tissue for substances coming from the circulation under the influence of a disturbance which brings about what is called an increased permeability of the meninges; and since fluid derived from each of these structures follows a different course within the central nervous system, it is to be expected that a substance delivered to the central nervous tissue from the circulation would depend for its distribution there upon whether it comes through one or another of these pathways. The criteria for affirming the passage from the circulation to the nervous tissue are the setting up of infection within the nervous system or the demonstration of the test substance in the cerebrospinal fluid, but in neither case may it be said with certainty through which gateway it has passed, and therefore the pathway which it has traversed within the central nervous system remains in doubt. While it is true in the case of infection with the virus of poliomyelitis that the process may be found within the nervous tissue itself, it does not follow that the infective agent was deposited there by the capillaries of that region, for indeed it has been shown that lesions are always present in the meninges [5] even very early in the disease, and that intraspinal inoculation causes infection, although the virus disappears rapidly from the subarachnoid space.[5,36] Thus, by extension (multiplication of the virus) it reaches the tissue spaces within the brain and cord. In the case of the appearance of test substances in the cerebrospinal fluid, it is to be recalled that the fluid as obtained by lumbar puncture represents the combined product of the choroid plexus and the perivascular system; hence the presence of a substance in this fluid gives no indication as to which one of the pathways it has traversed. Nor can it be supposed that a substance dissolved in cerebrospinal fluid would enter the substance of the central nervous system in a manner similar to that which may take place in the case of a multiplying virus.

The fluid elaborated by the choroid plexus into the lateral ventricles of the brain flows through the foramen of Monro to the third ventricle, then to the fourth ventricle and through the foramina of Magendie and Luschka to the subarachnoid space, from which it is finally drained into the dural sinuses through arachnoid villi and to a certain extent along the sheaths of cranial and spinal nerves, finally to be taken into lymphatic channels. Thus it would appear that that portion of the cerebrospinal fluid coming from the choroid plexus is never brought into intimate contact with the central nervous tissue itself but remains without the boundaries of the nervous structure, namely, the pia mater and the ependymal lining of the ventricular system. It follows, then, that immune serum coming from the circulation along with this fluid would have little chance of being brought into contact with poliomyelitic lesions within central nervous tissue.

In like manner any fluid discharged from the capillaries of the meninges would pass immediately to the fluid in the subarachnoid space to be carried away along with the fluid from the choroid plexus.

There remains to be considered that portion of the cerebrospinal fluid which is elaborated by the capillaries within the nervous tissue itself. This fluid passes through the walls of capillaries and circulates within the perivascular-perineuronal system, carrying nutritive substance to, and removing waste products from, the cells, and finally discharging into the subarachnoid space and becoming a part of the common cerebrospinal fluid. It is this fluid alone which bathes the cells of the central nervous tissue, and only through it can it be expected that immune serum would reach the cells of the nervous structure from the circulation. In order for such a pas-

sage of immunity principles to take place it would be necessary that the integrity of this capillary system be altered. As has been pointed out, the exact mechanism of the changes which permit the passage of immune bodies from the circulation to the cerebrospinal fluid involves especially the blood vessels of the meninges and then the choroid plexus. Increased permeability of the capillaries within the nervous tissue has not been demonstrated in this connection, and from the location of these capillaries with reference to the meningeal surfaces such an assumption is hardly justified, so that it may be considered highly improbable that these capillary walls are the gateway through which substances pass.

With these considerations in mind the statement may be made that the intravenous administration of immune serum for the treatment of acute poliomyelitis is beset with the following difficulties:

(a) The great dilution of the serum in the circulation renders only a small proportion of it available for passage to the site of the lesions.

(b) The barrier between the circulating blood and the central nervous tissue, the capillaries of the central nervous tissue itself, choroid plexus, and meningeal blood vessels would tend to reduce still further the proportion of serum which gains entrance to the central nervous tissue.

(c) Given the conditions under which immunity substances are permitted to enter the cerebrospinal fluid from the circulation, it is still, on account of the particular portion of the barrier which is broken down by the means at hand and the distribution within the central nervous system that it permits, highly improbable that they are brought into contact with the main lesions of acute poliomyelitis. In this connection it should be stated that, although the vessels of the nervous tissue are involved in poliomyelitis, no increase in their permeability has been demonstrated.

Intraspinal Serum Treatment

It has been shown by Weed [31] that true solutions introduced into the subarachnoid space fail to enter the perivascular system of the central nervous tissues but join the current of the fluid contained in this space which, as was pointed out in a previous paragraph, flows over the surfaces of the meninges and toward the exits from the central nervous system into dural sinuses. Clinically, the reaction of meningococcus lesions to immune serum introduced into the spinal subarachnoid space affords additional evidence of such a pathway. Lesions along the surface of the meninges (the typical meningitis) yield readily to serum administered in this way, whereas lesions within the nervous tissue, or in portions of the subarachnoid space, which are not in direct communication with that portion into which the serum has been introduced, do not respond to the same procedure.

Flexner and Amoss [24] in experiments upon the neutralization of the virus of poliomyelitis with immune serum found that, by the introduction of an immune serum into the subarachnoid spaces, the virus is capable of being neutralized within the cerebrospinal fluid into which it is directly introduced, or to which it passes in transit from the blood to the nervous tissues. In the latter case neutralization probably is effected at successive stages in the process of transit of the virus from the blood to the nervous tissues. They considered it highly probable that the neutralization was accomplished before any quantity of the virus became attached to the nervous tissues themselves, earlier experiments by Flexner and Lewis [25] having shown that even when minute amounts of the virus were inoculated intracerebrally, neutralization was accomplished with very great difficulty by intraspinal injections of immune serum. In other words, when once attached to the central nervous system tissue, the virus is not neutralized by subarachnoid injection.

In connection with this failure of neutralization within the brain substance, the experiments of Murphy and Sturm [26] upon the fate of tissue inoculated into the brain are perhaps of interest and emphasize the difference in reaction between the central nervous system tissue and its adjacent structures. They found that a transplantable mouse sarcoma grew readily when inoculated into the cerebrum of mice, provided the graft did not come in contact with the ventricle, in which case a cellular reaction occurred, similar to that about a subcutaneous heteroplastic graft. A bit of the animal's own spleen inoculated into the brain, along with the heteroplastic tumor tissue, prevented the growth of the foreign cells. Mice immunized to transplanted tumors were inoculated with grafts of mouse carcinoma, both subcutaneously and into the brain. Subcutaneous inoculation produced tumors in only 21 per cent of the animals, whereas the grafts into the brain produced tumors in 89 per cent. Control non-immunized mice inoculated with the same material developed brain tumors in 91.9 per cent and subcutaneous tumors in 82.2 per cent. Thus, mice highly resistant to subcutaneous transplants gave no evidence of this resistance when the tumor was inoculated into the nervous tissue of the brain.

There is now fairly complete evidence that the virus of poliomyelitis comes to be attached to a portion of the central nervous system which corresponds to that structural area into which immune bodies cannot be made to enter by either of the mechanisms of distribution referred to earlier in this paper. This seems to offer an explanation for at least a part of the lack of success with the serum treatment of this disease, and emphasizes the necessity of seeking other methods for obtaining a more adequate distribution of this serum within the central nervous system.

THE PASSAGE OF SUBSTANCES FROM THE SUBARACHNOID SPACE INTO THE PERIVASCULAR SYSTEM

Weed [21] found that solutions of potassium ferrocyanide and to a certain extent carbon granules in suspension, introduced into the subarachnoid space, pass into the perivascular system of the brain when this organ is rendered anemic. From this he reasoned that there must be suction of fluid from the subarachnoid space to fill the perivascular spaces and make up for the loss of fluid in the brain resulting from anemia. Thus, the flow of fluid within the perivascular spaces, which normally is toward the subarachnoid space, was reversed. The method employed for the production of the anemia was tying the carotids or exsanguination. The effects produced were attributed to changes in the osmotic relationship between the blood stream and the cerebrospinal fluid. Later, Weed and McKibben [23] found that the simpler procedure of injecting strongly hypertonic solutions, such as a 30% solution of sodium chloride, into the circulation produced a marked reduction in the cerebrospinal fluid pressure. This finding indicated that this procedure would also serve to reverse the direction of flow within the perivascular system, as Weed and Hughson [1] have shown that the reduction in the cerebrospinal fluid pressure is due not to increased absorption of fluid into the dural sinuses alone, but to a withdrawal of fluid from the tissue spaces into the capillaries. The addition of a foreign test solution to the subarachnoid fluid amply confirmed this point. When the cerebrospinal fluid pressure was falling rapidly or after it had become negative following the intravenous injection of hypertonic salt solution, two or three cubic centimetres of a potassium ferrocyanide and iron ammonium citrate solution were allowed to flow into the subarachnoid space. By subsequent fixation of the tissues in acidified formalin this solution was precipitated as Prussian blue at the points which it had reached. The solution was found to have passed from the subarachnoid space along the perivascular spaces reaching the interfibrous spaces in the white matter and the pericellular spaces in the grey matter.

These experiments, the general findings of which have been confirmed by numerous observers,[10,11,12,13,14,38] indicate that the intravenous injection of hypertonic solutions of sodium chloride brings about new ratios between secretion and absorption of cerebrospinal fluid and causes a dislocation of a considerable quantity of the contents of the subarachnoid space into the perivascular-perineuronal system. They suggested the possibility of obtaining a more effective therapy with convalescent serum in poliomyelitis by placing it in the subarachnoid space and subsequently injecting hypertonic solutions into the circulation.

EXPERIMENTAL

The alterations in the fluid circulation of the central nervous system which form the basis of our therapeutic experiments have been made only on normal animals. On account of the particular conditions which exist in those structures which are chiefly concerned in the alterations spoken of in the presence of poliomyelitis infection, there was some question as to whether the same alterations would be produced in the œdematous infiltrated tissues of this disease.

Exp. 1.—Macacus rhesus 27 was inoculated * intracerebrally with 2.5 c.c. of a suspension of glycerolated human spinal cord which contained the virus (Brow strain). On the ninth day after inoculation, the animal showed excitability, tremors, paralysis of the arms. On the following day the paralysis had increased in the arms and was beginning in both legs.

Injection experiment.—Under ether anesthesia (which was maintained at a constant level by insufflation from a Woulfe bottle which did not require changing during the course of the experiment) a lumbar puncture was performed and the needle at once connected with a U-shaped manometer filled to zero with

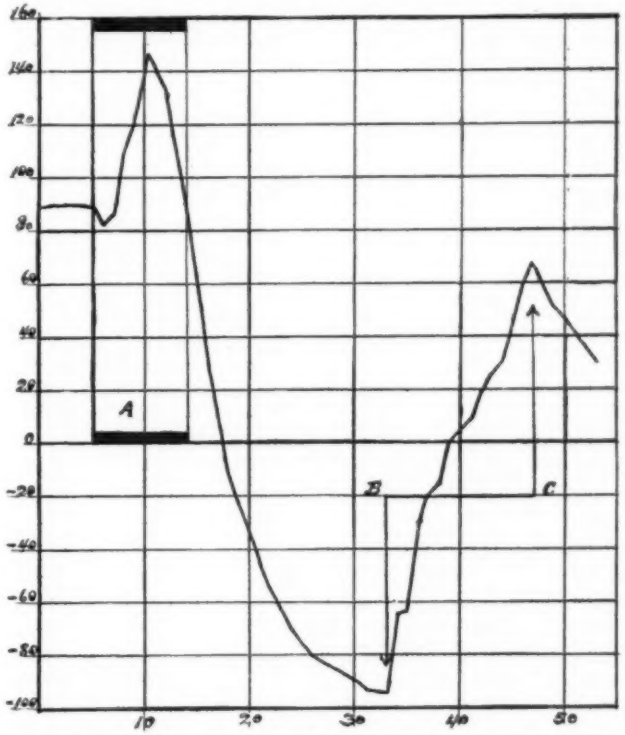

Chart 1. Experiment 1. Monkey 27 in acute stage of poliomyelitis. Weight 1320 grams. Ordinates represent cerebrospinal fluid pressure in millimetres of water; abscissae represent time in minutes. During blocked interval A, intravenous injection of 12 c.c. of 30% solution of sodium chloride. During the interval from B to C, introduction of 3.5 c.c. of a solution of 0.5% potassium ferrocyanide and 0.5% iron-ammonium citrate.

* All inoculations and other operative procedures were carried out under ether anaesthesia.

Ringer's solution. Cerebrospinal fluid pressure was recorded at one minute intervals throughout the experiment. After 5 minutes, during which the pressure ranged from 84 to 90 mm. of Ringer's solution, 30% sodium chloride was slowly injected intravenously. A total of 12 c.c. was given in 9 minutes. Within 4 minutes after the beginning of the intravenous injection the pressure rose to 146 mm. of Ringer's solution. It then fell rapidly to minus 94 mm. after 27 minutes from the beginning of the intravenous injection. At this point 3.5 c.c. of a solution of potassium ferrocyanide and iron ammonium citrate was introduced into the subarachnoid space through a burette attached to the manometer connection by means of a three-way cock. This solution was allowed to run in slowly during a period of 13 minutes. The addition of this amount of fluid caused the pressure to rise to plus 67 mm., after which it fell within the 6 minutes following to plus 30 mm., at which point the experiment was terminated. The tissue was fixed and the cyanide-citrate precipitated as Prussian blue by injection into the aorta of 10% formalin containing 1% hydrochloric acid. Microscopic examination of sections showed granules of dye distributed in the perivascular spaces. A full report of the distribution of the dye will be given in another communication.

Exp. 2. M. rhesus 23.

December 6, 1921. Inoculated intracerebrally with 0.5 c.c. 5% suspension of glycerolated spinal cord of Monkey 19.

December 17, 9 A. M. Apparent weakness of right hamstring. 8 P. M. Partial paralysis of extensors of right arm.

December 18, 10 A. M. Paralysis of right arm and right leg. Tremors. Staccato cry. 3 P. M. 7 c.c. 30% sodium chloride solution intravenously (1.05 grams sodium chloride per kilogram).

Decemeber 19, 9 A. M. Condition unchanged. 10 A. M. 6 c.c. 30% sodium chloride solution intravenously (.9 grams sodium chloride per kilogram). 5 P. M. Condition unchanged.

December 20, 9:30 A. M. Paralysis stationary in right leg. Right arm "improving." 2:30 P. M. 6 c.c. 30% sodium chloride solution intravenously (.9 grams sodium chloride per kilogram).

December 25, Slight Improvement.

The animal recovered with complete paralysis of the right leg and a slight involvement of right arm.

Exp. 3. M. rhesus 18, weight 2 kilograms.

December 6, 1921. Inoculated intracerebrally, right side, with 0.5 of a 5% suspension of glycerolated cord of Monkey 19.

December 13, 6 P. M. Excitable.

December 14, 9 A. M. Excitable, head tremors, ataxia. Right facial weakness. 11 A. M. Right facial paralysis. 2 P. M. Paralysis of extensor muscles of both arms. Weakness of flexors of right arm. 4 P. M. 5 c.c. 30% sodium chloride solution intravenously (.75 gram sodium chloride per kilogram).

December 15, 9 A. M. Paralysis of arms increased. 5:30 P. M. 6 c.c. 30% sodium chloride solution intravenously (.9 gram sodium chloride per kilogram).

December 16, Paralysis extended in legs. 10 A. M. 6 c.c. 30% sodium chloride intravenously. 5 P. M. condition unchanged.

December 17, 9 A. M. condition unchanged. 8 P. M. Paralysis has extended. 8:30 P. M. 2 c.c. convalescent serum intraspinally.

December 18, 9 A. M. Somewhat improved, general appearance better. 11:45 A. M. 4 c.c. 30% sodium chloride solution intravenously. 11:50 A.M. Respiration ceased.

Exp. 4. M. rhesus 5.

December 18, 1922. 11:45 A. M. Inoculated intracerebrally with 2 c.c. of a 10% suspension of glycerolated poliomyelitic human spinal cord (Brow strain). A needle introduced into the lumbar subarachnoid space just before the intracerebral injection yielded no fluid until approximately 1 c.c. of the virus had been introduced into the cranial cavity, after which it began to drop slowly. The animal recovered in a few minutes from the ether anaesthesia and during seven days following was entirely normal in appearance.

December 26, 9 A. M. Tremors of head. Ruffled coat, excitable. Paralysis of both arms, weakness of back and paralysis of adductors of right thigh.

12 M. Under ether anæsthesia. 20 c.c. of 30% solution of sodium chloride were injected intravenously at the rate of 1 c.c. per minute. Immediately after the beginning of the injection of sodium chloride solution, a needle was introduced into the lumbar subarachnoid space and connected to a manometer from which convalescent serum was allowed to flow into the subarachnoid space at a pressure which was not allowed to exceed 120 mm. of water. Fifteen minutes after the beginning of the injection of hypertonic salt solution the serum flowed quite rapidly into the subarachnoid space, this continuing for thirty minutes, at which time 10 c.c. had been used. The animal recovered from anæsthesia promptly and had a moderate chill. Within an hour, although still sluggish, was trying to sit up.

December 27, 9 A. M. No increase in paralysis. General appearance much better than before beginning of treatment. 12 M. Treatment was again undertaken, but after 12 c.c. of hypertonic salt solution had been given intravenously and 4 c.c. of serum intraspinally, respiration ceased.

Exp. 5. M. rhesus 29.

March 16, 1923. Inoculated intracerebrally with 1 c.c. of a 5% suspension of glycerolated brain and cord of Monkey 5.

March 30, 9 A. M. Excitable. Tremors. Weakness in both arms. 11 A. M. Paralysis of arms. Marked weakness in both legs. 2:50 P.M. Lumbar puncture was done and the needle connected with a burette-manometer. Cerebrospinal fluid pressure was recorded between 85 and 90 mm. water. 2 c.c. of human convalescent serum were allowed to flow into the subarachnoid space, which caused a rise in cerebrospinal fluid pressure

to 130 mm. water. Occipito-atlantoid puncture was then done and the cerebrospinal fluid allowed to drip until its pressure had fallen to 85 mm. water. The fluid from the occipito-atlantoid puncture was at first clear and later showed an admixture of serum. Ten cubic centimeters of 30% solution of sodium chloride were then given intravenously at the rate of 1 c.c. per minute. This caused a rapid reduction of cerebrospinal fluid pressure. At intervals, as the pressure fell, small amounts of serum were allowed to run into the subarachnoid space so that the cerebrospinal fluid pressure was not raised above 100 mm. water.

This animal recovered from the ether anæsthesia promptly but was quite sluggish the rest of the day.

March 31, Does not appear ill. Is alert and makes efforts to move about. No extension of paralysis.

April 1, General appearance better. Improvement in general appearance in this animal was prompt, but there was some paralysis in all extremities which persisted.

Exp. 6. M. rhesus 31. Weight 1420 grams.

April 7, 1923. Inoculated intracerebrally with 1 c.c. of a 5% suspension of the glycerolated cord of Monkey No. 5.

April 16, 8 A. M. Excitable. Ruffled coat. Head tremors, slight ataxia.
4 P. M. Same. No paralysis.

April 19, 8:30 A. M. Paralysis of extensor muscles of both arms and of both legs, more marked in right. 11:30 A. M. Occipito-atlantoid puncture. 3 c.c. of convalescent human serum introduced into cerebellar cistern, which caused the cerebrospinal fluid pressure to rise to 270 mm. water. This caused some respiratory embarrassment, which was relieved by lumbar puncture with a reduction of cerebrospinal fluid pressure to 115 mm. water. 13 c.c. of 30% sodium chloride solution intravenously at the rate of 2 c.c. per mm. Cerebrospinal fluid pressure rose to 165 mm. water and then fell rapidly to minus 16 mm. water. 7 c.c. convalescent serum slowly introduced into the cerebellar cistern. 4 P. M. Somewhat slow but gets about cage. 12 c.c. convalescent serum intravenously.

April 18, Sitting up. Appears bright. No extension in paralysis.

April 19, Improved. Gets about with ease.

May 17, Recovery with partial paralysis of right leg.

M. rhesus No. 30, Control for No. 31.

April 7, 1923. 3:15 P. M. Inoculated intracerebrally right side with 1 c.c. 5% suspension of glycerolated cord of Monkey No. 5.

April 17, 8 A. M. Excitable, marked head tremors, moderate ataxia. 9 P. M. Excitable, marked head tremors, more ataxic.

April 18, 9 A. M. Excitable, tremors, more ataxic; misses hold.

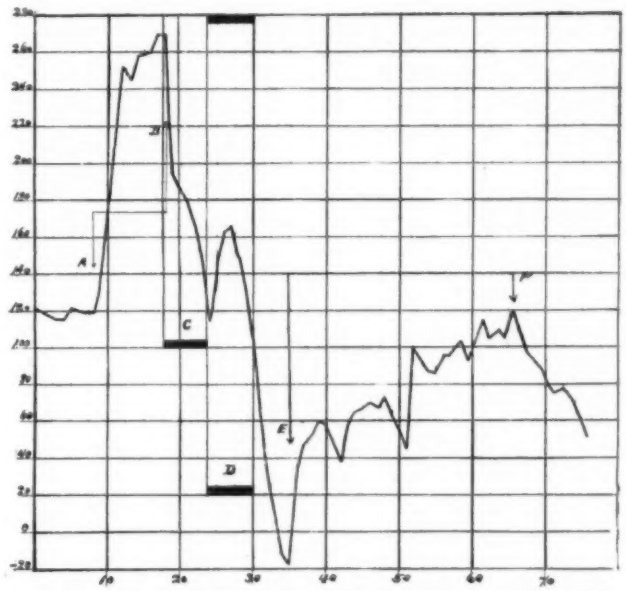

Chart 2. *Exp.* 6. Monkey 31, in acute stage of poliomyelitis. Ordinates represent cerebrospinal fluid pressure in millimetres of water; abscissae represent time in minutes. During the interval A to B 3 c.c. of convalescent human poliomyelitic serum were introduced into the cerebellar cistern. During the blocked interval C, cerebrospinal fluid was released from the subarachnoid space through lumbar puncture. During the blocked interval D, 13 c.c. of a 30% solution of sodium chloride were injected intravenously. During the interval E to F 7 c.c. of convalescent human poliomyelitic serum were introduced into the subarachnoid space.

April 19, 9 A. M. On floor. Flaccid paralysis of extensors of arms. Flexors good. Some weakness of legs.

April 20, Both legs flaccid.

April 21, Down, barely able to move arms. Appears ill; head tremors.

April 22, Paralysis same. Head tremors. Ill.

April 23, Paralysis same. Head tremors. Ill.

April 24, Paralysis increased. Almost prostrate. Head tremors.

April 25, Some oedema under eyes. Some tremors.

April 27, Same.

April 28, Same.

Gradual subsidence of acute symptoms. Unable to use extremities except hands. Gets about cage by pulling with hands and twisting body. Remained in same condition until May 17.

May 17, Sits up. Uses both arms and one leg fairly well.

SUMMARY AND DISCUSSION

The experimental findings presented indicate that the intravenous injection of strongly hypertonic solutions of sodium chloride causes marked alterations in the circulation of fluid within the central nervous system of monkeys in the acute stage of poliomyelitis (Exp. 1).

These alterations are apparently similar to those reported by other observers in normal animals, namely, reduction of cerebrospinal fluid pressure, reduction in the volume of the brain and spinal cord (removal of fluid) and a reversal of the current of fluid within the perivascular spaces.

Reduction in the volume of the brain and spinal cord suggested the use of hypertonic intravenous injections as a means of reducing to some extent the oedema of the central nervous system tissue in acute poliomyelitis. However, it is realized that factors other than osmosis are involved in edema. The course of the disease in one monkey treated in this manner (Exp. 2), as compared with the control animal in this series (Monkey 30) inoculated with the same strain of virus, is indicative of a beneficial effect of hypertonic solutions on the course of the disease.

Experiments 3 and 4 were designed to make use of the reduction in volume of the brain and spinal cord for lessening the oedema, as suggested by the previous experiment, and at the same time to replace the contents of the subarachnoid space with human convalescent serum which would be drawn into the perivascular spaces, under the influence of the hypertonic solution, and thus bring about a distribution of the serum, which does not take place after administration by other methods. In both these animals there was evidence of improvement following treatment, but unfortunately attempts to repeat the injection of relatively small amounts of hypertonic sodium chloride solution caused death of the animals.

Experiment 5 was carried out in the same manner as Experiments 3 and 4 except that only one injection was given. As shown on the protocol, this monkey had marked involvement when treated, but his condition on the following day was strikingly better. Recovery, although accompanied by a considerable degree of paralysis, was prompt as compared to the slow recovery of the control monkey 30.

Experiment 6 was a repetition of Experiment 5 with the addition of an intravenous injection of serum given 3 hours after the injection of hypertonic salt solution; in order that antibodies would be present in the blood when the flow of fluid from the circulation to the central nervous system fluid spaces was re-established. This animal showed an almost immediate improvement. There was no extension of paralysis after the one treatment, and recovery was rapid with only a partial paralysis of the right leg. As compared with the usual course of the disease in monkeys the outcome of this experiment is regarded as very favorable.

Conclusions

This small series of experiments indicate that the use of intravenous hypertonic solutions in conjunction with intraspinous convalescent serum has a favorable influence on the course of experimental poliomyelitis. This effect is augmented by the intravenous injection of convalescent serum at a time corresponding to the compensatory increase in passage of fluid from the circulation to the central nervous system tissue.

The optimal conditions in respect to the administration of hypertonic solutions remain to be worked out.

Daily repetition of hypertonic sodium chloride solution is associated with danger of respiratory failure.

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CLASMATOCYTES AND TUMOR CELLS IN CULTURES OF MOUSE SARCOMA[1]

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INTRODUCTION

The cultivation of both sarcoma and carcinoma has been carried on more or less successfully by many investigators during the past twelve years,[2] and it is now well established that sarcoma cells migrate out from the explants and behave much as do the connective-tissue cells; in fact, some of the figures of cultures of sarcoma resemble very closely the radiating reticular outgrowths of connective tissue. Carcinoma cells, on the other hand, as might be expected, migrate out in the form of sheets or membranes like those produced in cultures by normal epithelial cells. Both types of cells are actively amoeboid and mitoses have been frequently observed in them. No one, so far as we are aware, has called attention to the presence of clasmatocytes (resting wandering cells or macrophages) in cultures of sarcomata. Since these cells are markedly abundant in our cultures, it seems possible that they were present also in some of the cultures made by others but were not identified as such.

Lambert and Hanes (1911-13, p. 497-98), for example, recognized, in cultures of rat and mouse sarcoma, two fairly distinct morphological types of cells, a long spindle-shaped type and a second type which in actively growing cultures greatly predominated. The second type was characterized by its extreme irregularity in shape, due to the pseudopods which project from the cell in the most bizarre manner. They were inclined, however, to regard these two types as merely morphological variations and not generically separate types. The cells of the second type, shown in figure 5, resemble so closely, as does also the description, the clasmatocytes so abundant in our cultures, that it is not unlikely that the cells of this type were actually clasmatocytes. Since the sarcoma cells in our cultures were more distinctively different from the clasmatocytes than were the tumor cells from the sarcomata studied by Lambert and Hanes, we were able to distinguish more readily between the two types.

Practically all the cultures of tumor cells were made with plasma or modified plasma, until Drew (1922)[3] devised a saline solution in which calcium is present in the colloidal state. To this solution he added embryonic juice and was able to obtain continued growth of normal cells and tumor cells by subculturing. We have employed Drew's medium for the culturing of mouse sarcoma with considerable success and the following observations are all from cultures with the Drew saline-embryonic juice medium. Juice from the uterus and placentæ, when added to the saline solution, gave as good cultures as did the juice from the mouse embryos taken from the same uterus. So far we have not attempted subcultures, using only the original or primary cultures for observation.

OBSERVATIONS

Only two types of cells were observed migrating out from the explants. They were so strikingly different as to attract attention immediately. The first type corresponds in every particular to the ordinary clasmatocyte (resting wandering cell or macrophage) abundant in both embryonic and adult tissues. The second type represents the sarcoma cells proper.

In selecting the pieces of tumor from which to make the cultures we attempted to obtain tumor that was comparatively free from tissues of the host and as far away from any necrotic area as possible.

CLASMATOCYTES

Within an hour or two after the cultures were made the clasmatocytes, the first cells to appear, began to migrate out from the explant along the coverglass or on the surface membrane of the hanging drop. They were observed in all the cultures and there were usually many more of them than of tumor cells. They were also much more active and not only appeared first and moved faster than the tumor cells, but migrated farther out from the explant. Sometimes the periphery of the migrating zone was over two millimeters from the explant, and often the outer third or half of this consisted only of clasmatocytes (Figs. 1, 2), while the inner zone comprised both types of cells (Figs. 3, 4).

As might be expected, the active clasmatocytes were very irregular in outline (Figs. 1, 2, 5) and underwent

continual changes in form, while the inactive ones were more or less spherical (Fig. 6). One could watch the round and spindle-shaped forms elongate or become multipolar and vice versa. The irregular multipolar forms were the most common. The borders of these cells had a somewhat ragged appearance, due to the peculiar, more or less wavy, sheet-like processes attached here and there by curved lines to the coverglass. A superficial glance might readily lead one to believe that the processes consisted only of a slender curved attached part, but by careful focussing up and down one could follow what appeared to be a thin sheet hanging down between the two curved coverglass attachments. These wavy sheet-like processes in some instances extended from the under side of the cell directly into the fluid medium without coming into contact with the coverglass. Sometimes the entire periphery of the cell gave origin to such processes, which were a continuation of the thin cell edge. When the cells were irregularly multipolar in shape, these sheet-like processes were more abundant towards and at the ends of the poles (Fig. 5).

The clasmatocytes varied considerably in size (Fig. 6). This could be determined best by comparing the more or less rounded forms. The larger ones had more vacuoles and the differences in size were probably partly due to this. The clasmatocytes contained a small nucleus, usually eccentric, numerous fat globules of various sizes, granules and vacuoles which had an affinity for neutral red, and granular and short, rod-shaped mitochondria.

Tumor Cells

The tumor cells began to migrate some hours after the clasmatocytes and by 18 to 24 hours there were usually quite a number on the coverglass immediately around the explant. Within the explant they appeared to be round or oval in shape with an occasional short process. Along the edge of the explant (Fig. 7) the cells which were to migrate out became more elongated, sent out thin processes onto the coverglass from one or both of the thin, more or less flattened ends, and slowly moved out. When the cells first began to migrate, they were more or less spherical or oval and the processes were not very marked (Fig. 7). Later they became more elongated and irregular, and flattened out on the coverglass as large irregular, polygonal, triangular, or spindle-shaped forms (Figs. 3, 4). In extreme cases, as in Fig. 8, the tumor cells may become very much elongated.

The majority of the tumor cells contained a single, large, round or oval nucleus, homogeneous and waxy in appearance in the living cell. The nucleolus was fairly large. Binucleate cells were common (Fig. 3). Trinucleate cells were usually seen in every culture and occasionally even four nuclei were seen in a single cell. When two or more nuclei were present they were usually considerably smaller than the single ones.

The cytoplasm was unusually dense, not nearly so transparent as that of ordinary mesenchyme. This was due partly to their greater thickness and the large number of included granules. The cells stained very deeply with iodine, and janus green entered slowly, another indication of their density. The cytoplasmic inclusions consisted of numerous granular mitochondria, small granules and vacuoles which took up neutral red, ingested debris, and fat globules (Fig. 12). Lambert and Hanes (1911-13) have called attention to the phagocytic activity of tumor cells for carmine particles.

Even when the tumor cells were in the explant they contained both fat globules and a few small vacuoles and granules which took up the neutral red. As they migrated out, these became more evident (Fig. 7). On the second day they were still more marked (Figs. 9, 10), and by the 6th day many of the cells had become quite highly vacuolated (Fig. 11). These granules and vacuoles are probably identical with those which Lambert and Hanes (1911-13) observed in mouse sarcoma cells and called protoplasmic granules. They found that the granules became highly colored by carmine after the cells had phagocytized particles of the latter.

Discussion

Since spreads of the tumor at the time of planting showed numerous clasmatocytes, the latter were undoubtedly present when the tumors were dissected and cut up for cultures. The spreads were made by first immersing small pieces in neutral red-saline solution until the clasmatocytes had taken up the neutral red. The pieces were then pressed out under a coverglass and examined with the oil-immersion. As the numerous vacuoles in the clasmatocytes stain brilliantly with neutral red, they are easily seen in the spreads.

The question naturally arises as to the source of the clasmatocytes. Did they come from the host or were they produced by multiplication within the tumor as the latter grew in size within the host? Spreads from the subcutaneous tissue of the hosts showed many clasmatocytes, though they were not quite as numerous there as within the tumor. While spreads of the subcutaneous tissue and abdominal wall showed many clasmatocytes, only a very few migrated out in cultures of these tissues. Cultures of embryonic mouse mesenchyme, on the other hand, always showed numerous migrating clasmatocytes similar to those in the tumor cultures. When, for example, embryonic mouse heart and sarcoma were cultivated in the same culture, it was not possible to distinguish between the clasmatocytes arising from the two types of tissue. Why clasmatocytes migrate more readily from embryonic and sarcomatous tissues than from adult tissues is not clear.

Sections of tumor usually show numerous small nuclei scattered among the larger tumor-cell nuclei which we believe to be nuclei of the clasmatocytes. The cells are

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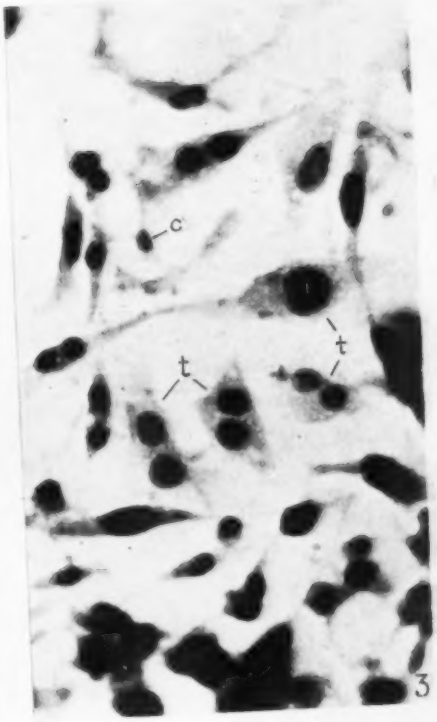

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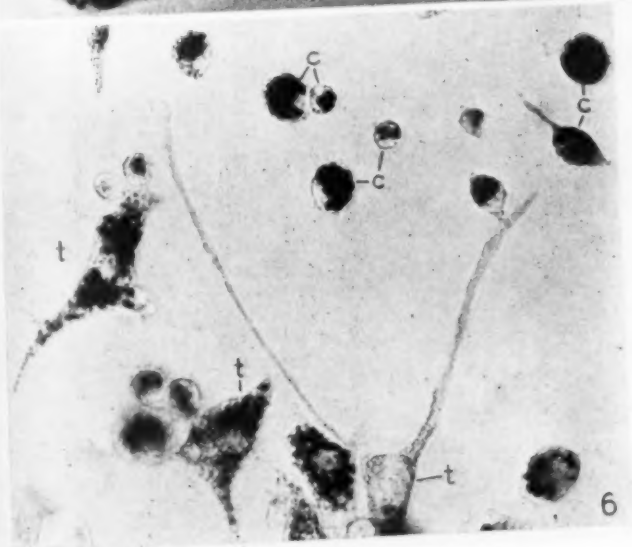

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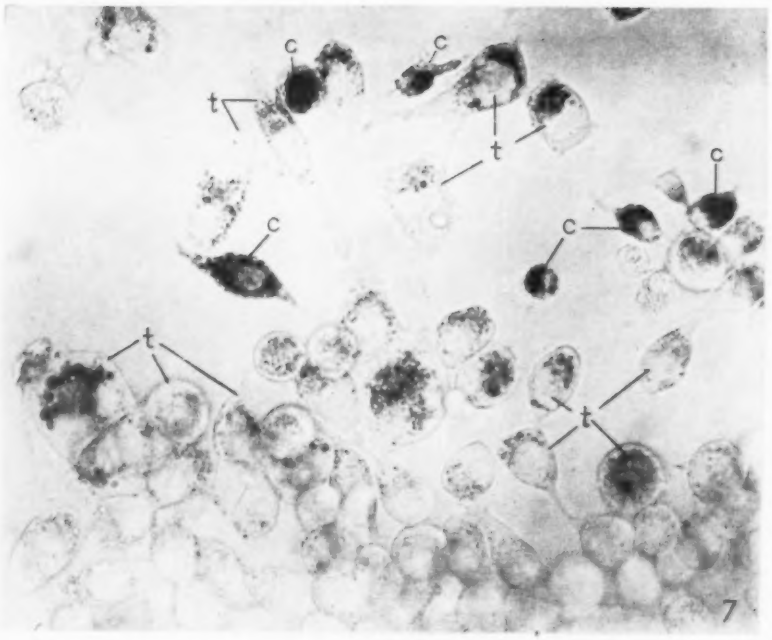
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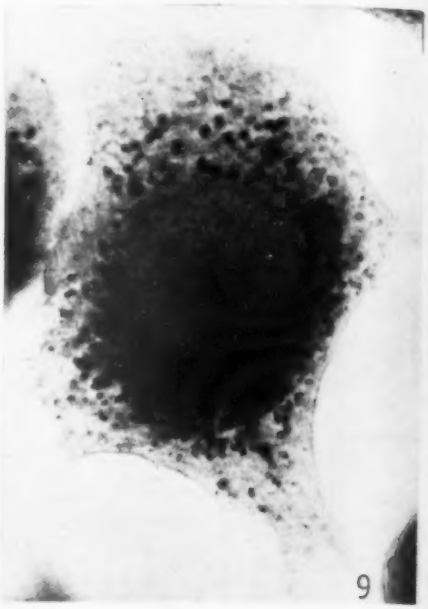
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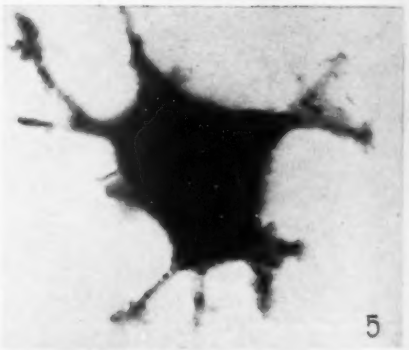
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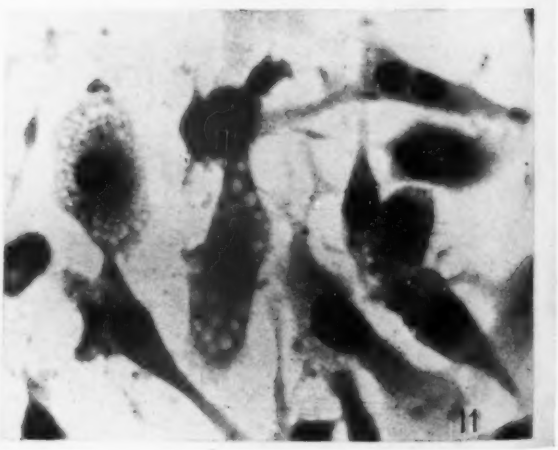
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so closely packed together that it is not possible to recognize the clasmatocytes by their cytoplasmic characteristics. Such texts as we have examined make no mention of the presence of clasmatocytes in sarcomata, and as the microscopical descriptions are based on sections, this is not surprising.

SUMMARY

Cultures of mouse sarcoma No. 180 in Drew saline-embryonic juice medium showed only two types of migrating cells in the primary cultures; namely, clasmatocytes and tumor cells.

The clasmatocytes were usually more abundant than the tumor cells. They began to migrate first and proceeded farther out on the coverglass than did the tumor cells. They were smaller and more irregular, with smaller nuclei and more vacuoles than the tumor cells.

The sarcoma cells, which were more or less round in the explants, elongated and became irregular in outline as they migrated and flattened out on the coverglass.

Both types of cells contained numerous fat globules of various sizes, granules and vacuoles which took up neutral red, and rather small granular and short, rod-shaped mitochondria. In the early stages the vacuoles are more conspicuous in the clasmatocytes, but in older cultures the tumor cells may become highly vacuolated.

EXPLANATION OF FIGURES

Fig. 1.—Clasmatocytes in the outer zone of migration. Two-day culture (913) sarcoma No. 180. Iodine vapor, hæmatoxylin, eosin. × 480.

Fig. 2.—Clasmatocytes (c) and few tumor cells (t) in the outer zone of migration. Two-day culture (911a) sarcoma No. 180. Zenker-acid, hæmatoxylin and eosin. × 480.

Fig. 3.—Clasmatocytes (c) and tumor cells (t) in inner zone of migration. Three-day culture (913a) sarcoma No. 180. Iodine, hæmatoxylin and eosin. × 480.

Fig. 4.—Clasmatocytes (c) and tumor cells (t) in the inner zone of migration. Three-day culture (914) sarcoma No. 180. Iodine, hæmatoxylin and eosin. × 480.

Fig. 5.—Clasmatocyte from two-day culture (903c) neutral red, iodine. × 1450.

Fig. 6.—Clasmatocytes (c) and tumor cells (t) from 23-hr. culture (901a); living culture with neutral red. × 480.

Fig. 7.—Clasmatocytes (c) and tumor cells (t) at the edge of the explant; 23-hr. culture (901); living culture with neutral red. × 480.

Fig. 8.—Clasmatocytes and one very much elongated tumor cell (.5 mm.). Six-day culture (910); living culture with neutral red. × 146.

Fig. 9.—Tumor cell with neutral red granules; debris granules dark. Two-day culture (903); neutral red and iodine. × 1450.

Fig. 10.—Tumor cell and clasmatocyte, same culture as figure 9 (903e). × 1450.

Fig. 11.—Vacuolated tumor cells. Six-day culture (910b); Zenker-acid, hæmatoxylin and eosin. × 480.

Fig. 12.—Tumor cells with fat globules. Two-day culture (912a); neutral formalin, hæmatoxylin and Biebrich' Scarlet. × 480.

FOOT NOTES

1. Dr. Francis C. Wood of the Institute of Cancer Research, Columbia University, very kindly sent us a mouse bearing the Crocker Fund tumor No. 180 from which all our material has subsequently been obtained. Mice have been inoculated every week or two and the material for cultures taken from them one to three weeks later.

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3. NaCl .900; KCl .042; $NaHCO_3$ .020; $CaCl_2$ .020; $CaH_4(PO_4)_2$ .010; $MgHPO_4$ .010; H_2O 100.

Stock solutions of each one are made up 10 times the final concentration. 10 c.c. of the NaCl, KCl and $MgHPO_4$ are then pipetted into a flask and 40 c.c. of distilled H_2O added. The calcium solutions and the bicarbonate are steamed 10 minutes (not autoclaved) in three separate flasks and just before being used 10 c.c. of each are pipetted into the mixture. The pH should be about 7.4. Two parts of embryonic extract are added to three parts of the saline solution.

THE JOHNS HOPKINS HOSPITAL BULLETIN

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A NOTE ON THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF MERCUROCHROME ON THE BACTERIOSTATIC ACTION OF BLOOD

By J. H. Hill and J. A. C. Colston

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In a preceding note,* we have reported the fact that, after single intravenous doses of from 1 to 10 mg/kg. of mercurochrome, urine becomes bacteriostatic, and in some cases bactericidal, for B. coli. We have also shown that increase in bacteriostatic action is not in direct proportion in urine to the amount of drug administered intravenously, such action being as marked following small doses as after large ones. The explanation for this finding probably lies in the fact that with the smaller doses, in rabbits from 1 to 5 mg/kg., the drug is eliminated largely through the kidneys, without marked intestinal disturbance, whereas with larger doses there is rapid elimination through the intestines.

Having found that such bacteriostatic action could be demonstrated in the urine, we have studied briefly the effect of the drug on the bacteriostatic action of blood, to determine whether or not such action was increased by the injection of mercurochrome and, if so, how soon and for how long after administration.

Method

Normal rabbits were injected intravenously in one ear with a freshly prepared solution of the drug. Single doses of 1, 2.5, 5 and 7.5 mg/kg. were given. One minute before injection and 15, 30, 45 and 60 minutes after injection measured specimens of blood were removed from the opposite, uninjected ear, brought to a total volume of 2 c.c. with sterile sodium citrate solution and inoculated with 1 standard loopful of a 24-hour beef-infusion (pH 7.6) broth culture of B. coli. Thirty-seconds after inoculation, 0.1 c.c. of the organism-blood mixture was removed, dilutions in 0.875% sodium chloride were made and plates poured to determine the number of organisms per cubic centimeter present at the time of inoculation. Similar specimens were removed, diluted and plated at intervals of from 1 to 24 hours after inoculation. The results so obtained are found in Tables 1 to 5. In these tables the figures in parentheses, obtained by dividing the number of bacteria obtained at a given interval of exposure by the number present at the beginning of the test, indicate the relative increase or decrease in the number of organisms.

* Hill, J. H. and J. A. C. Colston: A note on the bacteriostatic action of urine after the intravenous administration of mercurochrome to normal rabbits. Johns Hopkins Hosp. Bull., 1923, XXXIV, 220.

TABLE 1.
Rabbit 6.—One injection of 1 mg/kg.

Time of bleeding	Time of removal of specimens for plating and number of bacteria per c.c. so obtained			
	30 seconds after inoculation	1 hour after inoculation	24 hours after inoculation	Uninoculated control
1 min. before injection	4,900,000	5,400,000 (1.1)	151,000,000 (30.8)	0
15 min. after injection	6,900,000	6,400,000 (0.9)	85,000,000 (20.7)	0
30 min. after injection	4,300,000	.....	44,400,000 (10.3)	0
45 min. after injection	4,700,000	.....	126,000,000 (26.8)	0
1 hour after injection	5,100,000	.....	195,000,000 (38.2)	0

TABLE 2.
Rabbit 9.—One injection of 1 mg/kg.

Time of bleeding	Time of removal of specimens for plating and number of bacteria per c.c. so obtained			
	30 seconds after inoculation	2 hours after inoculation	24 hours after inoculation	Uninoculated control
1 min. before injection	6,900,000	3,900,000 (0.56)	15,264,000 (2.21)	0
15 min. after injection	7,400,000	7,000,000 (0.94)	6,900,000 (0.93)	0
30 min. after injection	6,800,000	5,100,000 (0.74)	2,544,000 (0.37)	0
45 min. after injection	6,700,000	6,100,000 (0.91)	7,632,000 (1.13)	0
1 hour after injection	6,100,000	6,000,000 (0.98)	15,900,000 (2.60)	0

TABLE 3.
Rabbit 7.—One dose of 2½ mg/kg.

Time of bleeding	Time of removal of specimens for plating and number of bacteria per c.c. so obtained		
	1 minute after inoculation	24 hours after inoculation	Uninoculated control
1 min. before injection	7,200,000	229,000,000 (31.8)	0
15 min. after injection	4,000,000	32,000,000 (8.0)	0
30 min. after injection	6,300,000	142,000,000 (22.54)	0
45 min. after injection	7,700,000	168,000,000 (21.81)	0
1 hour after injection	5,000,000	230,000,000 (46.)	0

TABLE 4.
Rabbit 10.—One dose of 5 mg/kg.

Time of bleeding	Time of removal of specimens for plating and number of bacteria per c.c. so obtained				
	30 seconds after inoculation	1 hour after inoculation	2 hours after inoculation	24 hours after inoculation	Uninoculated control
1 min. before injection	6,700,000	6,200,000 (0.92)	6,500,000 (0.97)	6,053,000 (0.90)	0
15 min. after injection	9,700,000	7,600,000 (0.78)	4,500,000 (0.46)	3,053,000 (0.31)	0
30 min. after injection	8,200,000	5,500,000 (0.67)	4,600,000 (0.56)	2,544,000 (0.31)	0
45 min. after injection	5,300,000	4,100,000 (0.77)	3,200,000 (0.60)	4,515,600 (0.85)	0
1 hour after injection	9,300,000	6,500,000 (0.69)	7,500,000 (0.80)	3,200,000 (0.34)	0

TABLE 5.
Rabbit 5.—One dose of 7.5 mg/kg.

Time of bleeding	Time of removal of specimens for plating and number of bacteria per c.c. so obtained		
	30 seconds after inoculation	24 hours after inoculation	Uninoculated control
1 min. before injection	1,160,000	16,300,000 (14.1)	0
15 min. after injection	940,000	13,200,000 (14.0)	0
30 min. after injection	1,250,000	14,300,000 (11.4)	0
45 min. after injection	2,700,000	2,800,000 (1.0)	0
1 hour after injection	2,100,000	8,400,000 (4.0)	0

SUMMARY OF FINDINGS

In these experiments it has been shown that, following the intravenous administration of mercurochrome, the bacteriostatic action of the blood against B. coli is increased. This increase appears to be greatest from 15 to 45 minutes after injection, after which it rapidly disappears.

REVERSIBLE GELATION IN LIVING CELLS

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Although cytologists have long been acquainted with the fact that certain parts of the cell may be more solid at one time than at another, and that the homogeneous cytoplasm and nucleoplasm become coagulated after various methods of fixation, only recently has any effort been made to interpret this phenomenon in terms of colloidal chemistry (Guaidukov, 1910; Chambers, 1917; Leblond, 1919; Bayliss, 1920; Taylor, 1923). Bayliss (1920) undertook to determine whether a reversible state of gelation could be induced at will by means of electrical stimulation in the homogeneous part of the pseudopodia of Amœba princeps. He found by the aid of the dark-field illumination that the Brownian movement of the minute particles made visible by this method ceased during the stimulation and was resumed as soon as the stimulation stopped. When it was found by the writer that a reversible coagulation of the living cell could also be brought about at will by means of acids, it seemed important to interpret this in terms of a reversible gelation.

The material used for the observations was the growth of connective tissue obtained in cultures from chick embryos (6 to 9 days' incubation). The tissue was explanted into Locke-Lewis solution (85 c.c. NaCl 0.9% plus KCl 0.042% plus $CaCl_2$ 0.025% plus $NaHCO_3$ 0.02% plus 15 c.c. chicken bouillon plus 1% dextrose) having a hydrogen-ion concentration between 6.8 and 7.1. Over five hundred cultures were utilized for the experiments. The acid solutions were prepared just before using by adding enough acid to the Locke-Lewis solution to make it pH 4.8, 4.6, 4.4, 4.2, 4, or 3.8, as the experiment required, and the alkaline ones were also prepared at the time of the experiment by adding alkali until the desired hydrogen-ion concentration (8, 8.6, 9 or 10 plus) was reached. The normal medium used for washing off was a standard one of pH 7.1 to which sodium hydroxide had been added to neutralize the bouillon when it was prepared. When acid or alkaline solution was placed on the cells, it was changed several times to obviate any dilution, and likewise, when washing off, the culture was bathed several times in order to remove all the acid or alkali.

These experiments, as well as a number of others not included in this publication, were made possible by a colorimetric method, carefully worked out by Dr. L. D. Felton (1921), for the determination of the hydrogen-ion concentration of such a small drop as a tissue-culture Without this valuable contribution much of the study of the chemistry of the living cell in tissue-cultures would be impossible.

Bayliss (1920) was able to determine whether the protoplasm of the amœba was in a gel state or a sol state by the immobility or activity of the minute particles visible by means of the dark-field illumination. In the cells in tissue-cultures, examined by W. H. Lewis (1923), there were no minute particles other than the mitochondria, neutral-red granules and fat droplets, visible in these cells even under intense dark-field illumination with a Zeiss baby arc, except under abnormal conditions, when very small white granules (death granules) appeared, both in the nucleus and in the cytoplasm. These granules exhibit Brownian movement only when in the cytoplasm,

never in the nucleus. Why no particles are visible in the protoplasm of cells in tissue-cultures is difficult to surmise. It may be that under normal conditions the particles are so small that they can be seen only after a certain amount of clotting has taken place, as Mathews (1920) describes for casein solutions. Or it may be that since these cells are spread out with so very much more thinness than can possibly be obtained with living cells by any other means, the particles may be so arranged that the light is not reflected from them. However that may be, it is interesting to note that when a cell undergoes mitosis during observation with the dark-field illumination, it becomes full of dancing light points as it rounds up and thickens for division, but loses them again as the daughter cells spread out over the coverglass after division. There was no need, however, to utilize the dark-field illumination for these experiments, as the coagulated state of the nucleus and cytoplasm was quite evident by means of the ordinary microscope. The earliest stage in the coagulation of the living cell occurs in the nucleus, in the form of very minute granules and an accentuation of the nuclear membrane. The nucleoli become clearer and brighter. This is followed by clotting of the nuclear substance into larger and larger granules with a brighter and thicker limiting membrane, until the nucleus becomes slightly shrunken and distorted into a coarse reticulum with here and there large masses of nuclear substance enclosed in a thick shiny membrane. The nucleoli may become embedded in some of the thicker parts of the clot, but even then they retain their identity. After this the cytoplasm begins to appear granular, at first slightly, then more coarsely so, with various threads and fibrils throughout the cell. As this takes place, the mitochondria become at first long and thin, then swollen and rounded, then varicose and beaded, and finally vesicular, or they may disappear entirely in the coagulum of the cytoplasm. The neutral-red granules and any vacuoles present lose their identity. Fat globules remain for some time; later some of these are extruded from the cell, forming very interesting long twists and curls, often of a beautiful, almost perfect pattern, as though they had been squeezed out. Frequently these twists seem to contain more material than was present in the original fat globule.

The coagulation of the cell may be brought about in many ways, but it is not always possible to reverse the phenomenon; neither does the cell always live, even after it has resumed its normal appearance. Coagulation brought about by means of various acids can always be reversed, provided the cell has not been killed. After the reversal of the gelation, the cells live and multiply almost as well as those of a normal culture.

The acids used in these experiments were sulphuric, hydrochloric, nitric, acetic, lactic, citric, oxalic, picric, and pyrogallic. All of these clotted the nucleus at about the same hydrogen-ion concentration (4.6) except pyrogallic acid, which, as has been described by Bauer, brought about this phenomenon at pH 6.4. With carbonic acid it was impossible to dissolve sufficient carbon dioxide in the medium to coagulate the nucleus.

The amount of acid necessary (pH 4.6) to produce an immediately perceptible, definite change in the nucleus is greater than that at which the cell can survive, for Lewis and Felton (1921) have shown that tissue seldom can survive in a medium containing more acid than pH 5.5, and that part of this acid is buffered by the explanted piece. Even solutions which do not contain sufficient acid (pH 5) to bring about an immediate change in the state of the nucleus, if not washed off, later cause coagulation of the nucleus and death of the culture.

When a culture was washed two or three times with a solution to which had just been added enough sulphuric, nitric, hydrochloric, acetic, lactic, citric, or oxalic acid to give it a pH of 4.6, the cells resembled those described as dead by Lewis and McCoy (1922). The nucleus was a mass of granules containing the nucleoli with a bright thick membrane around it. The cytoplasm remained homogeneous in appearance, the mitochondria were clear, rather long threads, some beginning to become swollen or rounded. All granules, especially the mitochondria, ceased their activity and remained more or less stationary. No Brownian movement was visible before the acid was applied and none became evident afterward, either in the nucleus or in the cytoplasm. All dividing cells remained in the stage of mitosis attained before the acid was placed on the culture. Even the clasmatocytes failed to send out or retract pseudopodia and became quiet. Whether there was any swelling of the cell due to the acid condition, as has been claimed by M. H. Fischer, could not be detected optically. If this solution was not washed off within the course of a few minutes, lines of stress began to appear in the cytoplasm, as though the processes were continued back into the cell body as fibrils. More fibrils appeared, the amount of homogeneous cytoplasm decreased, and the mitochondria began to round up and form vesicles. After the mitochondria have formed vesicles (not just rounded up), the cell is dead and no amount of washing off will cause it again to become alive, for although the coagulation of the cytoplasm may be removed to a large extent, that of the nucleus, especially the shiny membrane around it, usually remains. However, when the acid solution was washed off before the cytoplasm had become coagulated, the cell returned to its normal appearance; the nucleus and cytoplasm became homogeneous, the shiny membrane around the nucleus disappeared, the nucleoli became definite slightly opaque bodies, and the mitochondria and other granules in the homogeneous cytoplasm became discrete bodies and began once more to exhibit movement. The activity of the mitochondria was greatly increased. They bent, twisted, and moved from place to place more frequently than usual. Mitosis was often delayed for 10

or 15 minutes, but the cell completed the division and other cells that had not started to divide prior to the application of the acid began to undergo mitosis. If the culture was returned to the incubator, it lived as long and remained in as good a condition as the controls.

One of the most interesting features of the gelation phenomenon was the production of the spindle fibers in cells undergoing mitosis. In 1916 Lewis and Robertson showed that spindle fibers were not present in the living cell but that they could be formed through coagulation. To produce them the culture was suspended over acetic acid until fibrils appeared. No attempt was made at that time to reverse the gelation. In the present experiments it was found that acid solutions pH 4.6 or 4.4 caused the spindle fibers to appear as clear, shiny, straight threads. Also, in the early prophase, when the nuclear wall seemed to be dissolved, it became visible as a clear shiny membrane when acid was applied. These gelated structures disappeared when the acid was washed off and the spindle again became a homogeneous body, such as that figured in normal cultures by Levi (1916). Cell division was always delayed after this procedure but was usually completed. In most instances the early phases of mitosis were not as retarded as the later ones. In one instance, where a cell in early prophase lay near to one in late metaphase, the coagulation of the spindle and the nuclear wall was accomplished and reversed three times by means of an acetic acid solution of pH 4.4. After the acid had been washed off the third time, the cells did not complete division for over an hour, and then the cell which had been in prophase finished at about the same time as the one that had been in late metaphase, instead of about 20 minutes later, as is normal for these cells (Levi, 1916; Lewis and Lewis, 1917).

Gelation can be brought about and reversed several times in succession, but while these cells live and multiply they do not live as long or remain in as good condition as the controls or the cultures experimented upon only once. After gelation of the nucleus has been produced by an acid and reversed by washing off the acid, it can again be reversed without injury to the culture, even though brought about by a different acid. The more acid used, the fewer times can reversal of the gelation be accomplished and the more rapidly must it be washed off. Gelation brought about by solutions pH 3.8 can be reversed if immediately washed off, but with more acid than this many of the cells are immediately killed. When the cells are killed by the acid, the gelation becomes irreversible and cannot be removed by washing off the acid. The amount of acid necessary to bring about the immediate gelation of the cytoplasm was always more than that which coagulated the nucleus (being about pH 4.3) and after the cytoplasm had been coagulated it was proportionately more difficult to remove the gelation of the nucleus by washing with a normal solution; frequently, although the cytoplasm became homogeneous, the nucleus remained clotted and the cell was dead. In other words, the nucleus clotted upon the addition of much less acid than did the cytoplasm, but with a greater amount of acid the gelation of the nucleus was more difficult to reverse. When as much as 1% acid was placed on the cells both the cytoplasm and the nucleus became so coagulated that it was impossible to bring about the return of either to a homogeneous state. It was astonishing to how much acid the cells could be subjected in this rapid manner without distorting the mitochondria, while a smaller amount left upon the culture always resulted in the destruction of the mitochondria and the death of the cell. This was not due to the failure of the acid to penetrate the cell, for the state of the nucleus was sufficient evidence that it had passed through the cytoplasm.

In 1917 and 1920 the writer demonstrated that fibrils were not present in muscle cells grown in tissue-cultures but appeared upon fixation. This was also shown to be the case in the living heart of the chick embryo (M. R. Lewis, 1919). During the present study of the gelation phenomenon similar observations were made, acid solutions of pH 4.3 being used in place of fixing agents. The fibrils were produced by means of the acid and were removed by washing off the acid, provided the cells had not been killed. The heart of a 48-hour chick embryo was used, and, owing to the thickness of the cell layers, the penetration by the acid was much slower and often the cells of the outer layer of the heart were killed before the acid had reached the muscle. The heart continued to beat for some time but became quiet as the fibrils appeared. These were cross-striated as were the ones produced by fixing reagents. The nuclei were also coagulated. The fibrils and also the gelation of the nuclei were removed by washing out the acid. To accomplish this the heart was removed to a dish containing 10 c.c. of medium of pH 7.1 and allowed to remain there for a minute or so, when it was again placed on a slide with a coverslip over it for observation. In those instances in which the cells of the outer layer of the heart had not been killed, so that the nuclei resumed a homogeneous state, the heart again contracted and the fibrils disappeared; but where the nuclei of the outer cells remained coagulated, the heart did not beat again, even though the cells of the muscle layer lost their fibrils and their nuclei became homogeneous. When the muscle cells were killed by the acid, the fibrils remained and the nuclei did not become homogeneous when the acid was washed off.

Effect of Alkalies

The state of the normal fibroblast in tissue-cultures is probably semifluid, although practically no Brownian movement of any of the granules (mitochondria, neutral-red granules, or fat) is visible with the bright-field.

When cultures are to be observed by means of the dark-field illumination it becomes necessary to seal the growth in a thin film of fluid instead of leaving it in the hanging

drop. Under these conditions the granules usually exhibit Brownian movement (W. H. Lewis, 1923). Whether the difference in the behavior of the granules in the sealed preparation and in the hanging drop is due entirely to the fact that the dark-field illumination reveals this activity, or whether it is partly brought about by a change in the state of the cytoplasm due to the sealing of the culture, was not determined. The state of the cell could be made not only more solid, by means of acids, but also more fluid, by means of alkalies.

The fluid state was indicated by the cells beginning to round up instead of remaining spread out on the coverslip, by the mitochondria changing from long filaments to short rods or granules, and by a slight dancing of all the granules. These changes were followed by the appearance of fluid blebs which flowed out and in again along the edge of the cell. Finally, the whole cell became a round mass attached to the coverslip in the region of the nucleus, around which the cytoplasm kept flowing as one or more fluid, bleb-like pseudopodia, the particles of which exhibited Brownian movement. The fluid condition of the cells was produced not only by means of ammonia vapor, ammonium hydrate, and ammonium carbonate, but also by sodium hydroxide, potassium hydroxide, lithium carbonate, and sodium bicarbonate. The hydrogen-ion concentration of the solution of the different alkalies which brought about this phenomenon varied greatly, and it has not yet been possible to standardize these results. Usually a solution pH 8.6 to 9 caused fluidity of the cytoplasm, either slight or marked, when placed upon the cells. This could be reversed if the culture was bathed with a normal solution. Although the cell never spread out again, immediately after the alkali was removed the rounding up ceased, the blebs no longer flowed out, and all Brownian movement of granules or particles stopped. Those cells in mitosis completed the division, other cells divided, cells migrated out from the explant, and the culture after being returned to the incubator lived, though not nearly so long as the controls. Those cells which had become entirely round seldom spread out again but usually degenerated. When the amount of alkali was increased to pH 10 the nucleus, especially the nucleolus, dissolved. Sodium bicarbonate, however, often brought about the disappearance of the nucleoli in freshly prepared solutions of only pH 7.4 or 7.6. After the nucleus and nucleolus had disappeared they could be brought back by washing off the alkali, but in this reversal the nucleus frequently became slightly clotted instead of homogeneous. After the nucleoli had been dissolved by these alkalies the cells never lived. It was often possible to bring about the solution of the nucleoli before the cells rounded up by placing upon the cell a solution containing slightly more alkali than is necessary to dissolve the nucleoli. Nevertheless, these cells practically always rounded up after the alkali was washed off. The death of the cell was not due to the solution of the nucleoli alone, for a complete disappearance of both the nuclear wall and the nucleoli can be brought about by means of a strong dextrose solution and reversed by washing off the dextrose without resulting in the death of the cell. Much less alkali was necessary to bring about a fluid state of the cytoplasm than of the nucleus.

Discussion

It is evident from these experiments that the nuclear substance is different from that of the cytoplasm. It is more readily coagulated than the cytoplasm and by smaller amounts of acid; on the other hand, the cytoplasm is more rapidly dissolved by an alkali than is the nucleus. The reversal of the gelation of the nucleus can be readily accomplished and the cells continue to live, but the reversal of the gelation of the cytoplasm is more difficult and the cells seldom live. When the cytoplasm has been made more fluid by means of an alkali it can be returned to a gelatinous state, and in some instances the cells recover, but after the solution of the nucleus by means of an alkali the cells never recover, even though the nucleus and cytoplasm become gelated upon removal of the alkali.

Loeb and Blanchard (1922) showed that the alkalinity or acidity of the medium affects the state of the cytoplasm of the leucocytes. In more alkaline solutions leucocytes adhere to the glass and disintegrate, while in more acid solutions their consistency is so great that they cannot move. I find that this is especially true of leucocytes studied by means of sealed films. Under such conditions, white blood cells become distinctly fluid and the granules exhibit marked Brownian movement. This fluid cytoplasm flows about much more actively than do the firmer pseudopodia of the normal white blood cell.

In regard to the contractions of the heart muscle, Clark (1913) and Dale and Thacher (1914) have likewise demonstrated that the heart of the frog ceases to beat in slightly acid solutions, while Clark (1913) has shown that alkaline solutions pH 10 are fatal to it. Loeb (1915 and 1923) used the paralysis of the heart of fundulus to show that acid had penetrated the egg membrane.

It has not been possible for me to harmonize these results with the laws concerning the behavior of proteins established by Jacques Loeb (1922) from studies carried out on gelatin, because while it is a simple matter to determine the hydrogen-ion concentration of tissue-cultures during the growth, degeneration, and death of the cell, and also to find the range of alkalinity or acidity of the solution in which it is possible to obtain growth of tissues, it is impossible, so far as I know, to determine the hydrogen-ion concentration of the living cell. Vital dyes stain only certain definite structures of the living cell, and they enter the cell and stain these structures in solutions varying from slightly acid pH 4.8 to strongly alkaline pH 9. The color of janus green or brilliant

cresyl blue does not vary within this range, and while neutral red does, it furnishes data only in regard to the nature of certain preformed bodies existing within the cells (Fischel, 1901; M. R. Lewis, 1917; W. H. Lewis, 1919; Prigosen, 1921), and the resulting color in these bodies depends upon the chemical reaction of the neutral red solution (i.e., the acid or alkali added) and the substances existing in the bodies (CO_2, ammonia, amino acids, etc.). The same reasoning must also apply to the results obtained by means of natural indicators such as the color of flowers. While any change in the color of the flower certainly indicates that the substance used has passed into the cell, it does not necessarily determine the hydrogen-ion concentration of the living cytoplasm of the cell.

When the indicators most commonly employed in determining the pH of small quantities of material (Felton, 1921) were placed upon the cells, they afforded information only in regard to dead or dying cells, as they either did not color the living cell or else brought about the death of the cell, in which case its color became such as to indicate pH 6. Since the alcoholic solutions of the indicators always killed the cells, the indicator (in powder form) was dissolved directly in the nutrient medium and a drop of this placed upon the culture. The results were as follows:

Methyl red (Orthocarboxybenzene azodimethylaniline pH 4.4 to 5.6) formed a yellow solution in which the cells remained alive. The mitochondria were active and any vacuoles or granules present behaved as usual. No trace of color appeared in the living cell or any of its granules. The dead cells and debris stained yellow or a pale pinkish yellow. The nucleus was the same color as the cytoplasm but a deeper shade, as though there more material was stained.

Brom cresol purple (Dibrom-cresolsulphonephthalein pH 5.8 to 6.8) formed a reddish purple solution in which the cells lived. They remained transparent without a trace of color in the cytoplasm or nucleus. Debris and dead cells became reddish yellow.

Brom thymol blue (Dibrom-thymolsulphonephthalein pH 6.4 to 7.6) formed a pale green solution. The cells began to die quickly, so that along the edge of the growth the cells were all yellow within five minutes. The mitochondria disappeared almost at once and the cytoplasm and nucleus became yellow. Fat globules remained uncolored in the yellow cytoplasm.

Phenol red (Phenolsulphonephthalein pH 7 to 8.2) caused the solution to become an orange color. The cells remained alive and uncolored, while the debris became yellow.

Cresol red (O-cresolsulphonephthalein pH 7.8 to 8.4) formed a yellow solution and did not kill the cells. The cells remained uncolored, while debris became yellow.

Thymol blue (Thymolsulphonephthalein pH 8.6 to 9.2) also formed a yellow solution but was quite toxic. The cells remained colorless for from one to five minutes, but the mitochondria began to be dissolved out and had disappeared before the cells began to stain. The cells became yellow, the nucleus and nucleolus the same color but a deeper shade. Debris was also yellow. Within from 15 to 20 minutes all the cells were yellow and dead.

Thus it is clear that these indicators serve only to show that dead embryonic connective-tissue cells are slightly acid, about pH 6.

Acid fuchsin also afforded no results in regard to the living cytoplasm and the data obtained from fixed and stained tissue have bearing only upon the nature of the compound formed by the fixing agent with the cytoplasmic colloid or the nuclear colloid.

It is not surprising that it is impossible to determine the hydrogen-ion concentration of the living cytoplasm and nucleus by a color indicator, since the color change which takes place in an indicator is brought about by a rearrangement of the molecule to a colored form, probably due to the dissociation of the molecule. It is most unlikely that living cytoplasm could in this way be formed into a colored compound without bringing about sufficient change to cause its death. In 1919 W. H. Lewis expressed this same idea in regard to the action of vital dyes and stated that the particles in the cell which become stained are probably not a part of the living cytoplasm. Bechhold (1919) also states that such foreign bodies as become chemically fixed permanently injure the affected cell. Physiologists seldom take into account the fact that when a cell is living it is in a state of which almost nothing is known at the present time. Mathews, in the last edition (1920) of his book on Physiological Chemistry, emphasizes the remarkable nature of the living state and frequently warns the investigator not to be too hasty in applying results obtained from nonliving material. In one place he very aptly states: "The plant cell is not a bag of liquid with a semipermeable wall but probably a jelly-like substance. Furthermore, this gel is one of the most unstable substances known. It is living matter."

Some investigators have taken it for granted that the tissue has the same pH as the plasma surrounding it, but there is no proof of this nor is there any reason to suppose that the plasma itself may not become changed upon removal from the living animal or upon clotting. This is certainly true in regard to the sap of many plants. The pH of the plasma or hanging drop of medium of a tissue culture changes according to the activity of the cells, and also their behavior changes in media of different composition with a resulting difference in the pH of the medium; and yet it is impossible to measure the hydrogen-ion concentration of these cells. In addition to this, the experiments of Jacobs (1923) show that, even when a substance does penetrate a plant cell or an egg, the resulting color change of the natural indicator of the flower or of the body stained with neutral red in the egg can by no means be predicted from the pH of the medium.

From the results obtained when dilute acid or alkali solutions are placed upon the living cell it is evident that the theory regarding the action of the cell membrane in preventing the entrance of certain substances into the cell does not apply to connective-tissue cells of the chick

embryo.[1] This theory was formulated largely upon results obtained (Mathews, 1907, Warburg, 1910, Harvey, 1911, Lillie, 1912, Osterhout, 1914, Loeb, 1915, and others) from experiments on paramœcium, plant cells or various eggs, which cells undoubtedly do possess a definite membrane; but while the theory may be true in regard to these cells,[2] it cannot be applied *a priori* to other kinds of cells. The statement of Mathews (1907) that the nucleolus of the unfertilized egg is dissolved by means of ammonium hydrate but not by other alkali should be limited to the material which he used. This would also hold true in the interesting observations of Jacobs (1920, '22 and '23), for when human leucocytes are mixed half and half with ammonium chloride in Locke-Lewis solution pH 6.4 (NH_4Cl NH_4OH+HCl), the nucleus and nucleolus become dissolved[3] and the cytoplasm fluid, as though the cells were in a solution pH 10; but if the same experiment be carried out by adding HCl to the medium (pH 4), the nucleus becomes gelated and the cells partially or wholly paralyzed, showing that the HCl did enter these cells.

It is astonishing that the nuclear colloid can be separated by clotting into regions of more or less concentration without bringing about an immediate irrecoverable injury. In the reversible gelation the action of the acid is probably so inhibited or so weak that no foreign compound is formed, for when the acid is left on or sufficient acid used, the coagulation becomes irreversible and the cell is dead.

The ingestion of substances known to be colloidal solutions, such as trypan blue and white of egg (M. R. Lewis, 1921), and the segregation of these substances into large and small particles have been puzzling investigators for some time. Evans goes so far as to formulate the theory that the ultramicroscopic particles are phagocytized and segregated into definite vacuoles. That there is no such segregation apparatus in the normal fibroblast or human leucocyte has been amply proved by observations on living cells (M. R. Lewis, 1917; W. H. Lewis, 1919; Prigosen, 1921; Smith, 1921); nevertheless, foreign particles such as carbon, melanin granules, and even bacteria frequently become clumped in the living cytoplasm and often a fluid vacuole is later formed around them. This vacuole is not primarily a digestive vacuole, for it has been found that the digestion of particles takes place more frequently and more rapidly in the homogeneous cytoplasm than in vacuoles, even when vacuoles are present (M. R. Lewis, 1923). All these phenomena are undoubtedly related to gelation and solution phenomena of colloids. Such a solution as trypan blue undoubtedly enters the cytoplasm as ultramicroscopic particles which probably become clumped into larger particles by some action of the cytoplasm. This seems to be the mechanism by which albumin and plasma granules appear in cells which are grown in media containing these substances (M. R. Lewis 1921).[4]

One of the most effective acids in bringing about the gelation of the living cells was that obtained from sterile dead tissue which had stood for 20 hours in Locke-Lewis solution. Gelation produced by this means could also be removed by washing off the solution, but when it was not washed off the cells died. This suggests that part of the toxic nature of crushed or burned tissue may be due to this factor.

[1] In a separate communication it will be shown from the results obtained by the use of vital dyes that these cells are penetrated not only by carbon dioxide and ammonia but also by other acids and other alkalies.

[2] The statements of M. Irwin (1923) in regard to the permeability of living cells to dyes as affected by hydrogen-ion concentration cannot be applied even to plant tissue in general, because when the fluid is removed from the vacuole of the living cells of certain plants it undergoes so many changes due to difference in oxidation, etc., that it can no longer be compared with the substance existing in the living cell. The color which the bodies in the living cell become when stained with vital dyes, especially brilliant cresyl blue, depends upon the state of the cell (Lewis, 1923).

[3] It requires a very strong solution of NH_4Cl in Locke-Lewis solution to give an acid reaction.

[4] If the supposition that a virus is some sort of a continuous protoplasm, i.e., a colloidal solution capable of increasing itself, then it is quite possible that such a foreign substance might become clotted into visible particles (such as vaccine bodies, etc.) *in vitro* or after it has entered the cell. On the other hand, there is no reason to suppose that it may not remain invisible in the cell or in the plasma, just as do innumerable other substances. It seems to me that very careful observation of the behavior of the cells in the presence of known viruses must be made before any conclusion can be drawn in regard to the specific nature of bodies which appear in cells under abnormal conditions.

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OBSERVATIONS ON STRAINS OF HEMOLYTIC STREPTOCOCCI RECOVERED FROM CARRIERS AND CASES DURING AN OUTBREAK OF ACUTE TONSILLITIS*

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The objects, from the standpoint of clinical and epidemiological study, of dividing races of bacteria into definable sub-groups are twofold. It is necessary first to determine the number of types of an organism which may act as a unit in the epidemic spread of a disease, and secondly, if the course of dissemination of an infection through a group or community is to be followed accurately, some reliable means of recognizing a given strain of the organism as it appears here and there must be available. For the latter purpose it would be desirable to have a method whereby each strain, for example, of B. typhosus, or each strain of Pneumococcus Type I, could be differentiated from every other member of the same group.

In previous papers [1] we have reported observations on an outbreak of acute tonsillitis which occurred in a limited group of people during the winter of 1922-23. During the course of this study a large number of strains of hemolytic streptococci were recovered from the tonsils of carriers and from cases of acute tonsillitis. In the present communication we wish to describe the characteristics of these organisms. It was our purpose to ascertain whether a single strain of hemolytic streptococcus was responsible for all the cases in the outbreak, or whether a variety of organisms were concerned, and also to relate the case and carrier strains as far as possible.

* This is the fifth of a series of papers on streptococcus infection with special reference to acute tonsillitis.

The following methods of classification were available and received consideration.

1. Appearance on the blood-agar plate.
2. Fermentation of sugars.
3. Final hydrogen-ion concentration.
4. Iso-electric point (acid agglutination).
5. Agglutination by immune sera.
6. Complement fixation.

1. *Appearance on the blood-agar plate.*—This was employed as the initial criterion for classification. The directions given by Brown [2] were followed and special stress was placed on the use of poured plates of uniform thickness. It will be pointed out below that all the strains isolated from cases of tonsillitis showed the typical beta-hemolysis. No subdivision of groups was possible, however, by this method, despite considerable variation in the original appearance of the strains from various patients.

2. *Fermentation of sugars.*—Fermentation of sugars by the various strains of hemolytic streptococcus was tested in the usual way. Beef infusion nutrient broth was rendered sugar-free by the B. coli method, and was adjusted to pH 7.4. Sterile solutions of salicin, mannite, and lactose were added, so that the final sugar concentration was 0.1%. Phenol red was added as indicator. After pipetting 3 c.c. portions under sterile precautions into small test-tubes and incubating, the media were ready for use. Small amounts of media were used, so that the size of the inoculum would be relatively great, insuring heavier bacterial growth. Although the employment of still other carbohydrates might have served further to differentiate an occasional strain, it was felt that for the sake of simplicity it was better to work with only the three commonly used sugars: mannite, salicin, and lactose. After incubation of the cultures for five days at 37°, the reactions were read. Where fermentation had taken place, a sharp change in the color of the indicator was observed. No tube was read unless there was a marked macroscopic growth, and all irregularly fermenting strains were re-tested.

3. *Final hydrogen-ion concentration of cultures.*—There are many observations in the literature which show that the final pH of a culture grown under constant conditions is quite uniform, and is characteristic of the organism concerned. Arnold [3] has recently reviewed the subject and no further reference is necessary save to the paper of Jones,[4] who found that great variations in acid end-point resulted from changing the composition of the medium and its initial pH.

The following technique was used in the present work: To beef infusion broth made sugar-free by B. coli, dextrose was added to a concentration of 1% and the medium was finally adjusted to pH 7.3. In confirmation of Jones' work, we found that variations in the initial hydrogen-ion concentration materially affected the final pH of the culture media, so that a culture starting at pH 7.8 would fail to reach an end-point as acid as one whose initial pH was 7.2—7.3. Broth of pH 7.3 gave a maximum growth and uniform results, and was therefore used throughout these experiments. Two test-tubes containing 10 c.c. portions of the medium were heavily seeded, each receiving half of the growth from a 24-hour blood-agar slant. They were then incubated at 37° for 60 hours. After 24 hours, the tubes were well shaken to insure a uniform distribution of acid and bacteria throughout the medium.

All determinations were made in duplicate, and when the variation between the two readings was pH 0.10 or greater, the test was repeated. In other cases, where the variation was pH 0.10 or less, the lower pH reading was accepted as the correct end-point. However, in the great majority of cases there was a striking agreement in the two readings which rarely differed by more than pH 0.05.

"Universal Buffer Solution" * was used for making the colorimetric standard solutions, with methyl red as indicator. While they were not checked electrometrically, all standards throughout the series of experiments were made from a single stock preparation, and the normality of the hydrochloric acid used was repeatedly determined.

Another check on the accuracy of the method was the comparison of the final pH of the same strain determined at different times with different series of standards; the results were always in absolute or close agreement.

After sixty hours' growth, the supernatant was pipetted off, leaving behind the sedimented bacteria. After dilution with an equal part of distilled water, the pH of the medium was determined colorimetrically in a Walpole comparator.

To determine the constancy of the final reaction under the prescribed conditions, the acid end-points of five tonsillitis strains of hemolytic streptococcus were tested in duplicate on three successive days, with the following results:

TABLE I.

Experiment to test constancy of final pH of cultures by repeated determinations over a period of several days.

Strain	Dec. 16	Dec. 17	Dec. 18
	pH	pH	pH
S 118	5.50	5.47	5.41
	5.50	5.47	5.41
S 177 R T	5.42	5.45	5.4
	5.4	5.45	5.4
S 130 P	5.4	5.45	5.35
	5.4	5.43	5.40
S 177 L T	5.45	5.42	5.42
	5.45	5.42	5.38
S 180	5.45	5.40	5.40
	5.45	5.45	5.45

* Prepared by the Pyrolectric Instrument Company, Trenton, New Jersey.

The greatest variation in six determinations on a single strain is pH 0.09. The close agreement in the end-points of most of the tonsillitis strains is pointed out elsewhere in this paper. Avery and Cullen [5] showed that, when the tests were performed under uniform conditions, duplicate observations did not vary by more than pH 0.10. We have assumed, therefore, that where the lowest reading on a strain differed by pH 0.20 from the highest reading on another strain, the two might be regarded as different strains.

4. *Acid agglutination.*—In view of the recent observations of De Kruif [6] on the differentiation of two mutants of a single strain of the bacillus of rabbit septicemia by acid agglutination, it was hoped that this method might serve to separate various strains of hemolytic streptococcus isolated from carriers and from cases of streptococcus infection. Following the work of Michaelis,[7] Beniasch [8] carried out an extensive series of investigations on the acid agglutination of various bacteria, among which were nine strains of streptococcus pyogenes, and five strains of streptococcus isolated from scarlet fever cases. Using a sodium lactate-lactic acid buffer series, he found the agglutination range of all strains to be within a limit of C_H 5.5×10^{-4} (pH 3.26) and C_H 8.8×10^{-3} (pH 2.06) with an optimum of C_H 4.4×10^{-3} (pH 2.36), De Kruif [9] has shown that the acid agglutination optimum of the bacteria which he studied varies in some measure with the nature of the buffer mixture. His observations indicate also that the presence of traces of protein, peptone, electrolytes, etc., in media, affect the acid agglutination zone by broadening it or causing it to shift in the direction of a higher or lower C_H, and he points out the necessity of washing the organisms thoroughly to avoid the disturbing influence of these factors.

The following technique was therefore employed:—Mass cultures made on plain beef infusion agar (pH 7.4) plates were scraped off and washed four times by centrifuging and resuspending in redistilled water. Finally, the suspensions were brought to a standard turbidity, arbitrarily chosen, by means of a wire nephelometer similar to that described by Gates.[10] In certain cases, the granular character of the growth on agar could not be overcome and an even bacterial suspension could not be obtained, though occasionally repeated washings transformed a coarsely granular material into a diffuse suspension. The acid buffer series was prepared from the "Universal Buffer Solution" above referred to, a range from pH 2.4 to pH 5.7 being used, with intervals of pH 0.3. Equal parts of the standard bacterial suspension and of buffer were mixed and allowed to stand at room temperature. Readings were made at intervals up to twelve hours.

A group of streptococcus strains most of which were isolated from cases of acute tonsillitis was studied in respect to acid agglutination.

TABLE II.

Acid agglutination of 16 strains of hemolytic streptococcus.

Interval before reading	Strain	Source	Acid agglutination range in terms of pH											Fermentation reactions			Acid end-point (pH)
			2.4	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	5.4	5.7	Salicin	Mannite	Lactose	
2 hr.	180 LT	Tonsillitis	0	++	++	++	+++	+++	++	+	+	0	0	0	+	0	+	5.45
12 hr.			0	++	+++	++++	+++	+++	+++	++	+	0	0	0				
2 hr.	180 P	Tonsillitis	0	+	++	+++	+++	++++	+	+	0	0	0	0	+	0	+	5.45
12 hr.			0	+++	+++	++++	+++	+++	++	+	+	0	0	0				
2 hr.	130 P	Tonsillitis	0	+	+	++	+++	++++	+++	+	0	0	0	0	+	0	+	5.40
12 hr.			0	0	+	+	+++	+++	+	0	0	0	0	0				
2 hr.	190 LT	Carrier	0	0	+	+	+	++	+	+	+	+	+	+	+	0	+	5.10
12 hr.			0	+	+	++	++	++	++	++	+	0	0	0				
2 hr.	202 LT	Tonsillitis	0	+	+	+	+++	+++	+	+	0	0	0	0	+	0	+	5.40
12 hr.			0	+	+	+	++	+++	0	0	0	0	0	0				
2 hr.	166	Carrier	0	+	+	+	++	+++	+	+	0	0	0	0	+	0	+	5.5
12 hr.			0	0	0	0	+	++++	++	+	0	0	0	0				
2 hr.	199	Tonsillitis	0	+	+	+	++	++	++	0	0	0	0	0	+	0	+	5.15
12 hr.			0	+	++	+++	+++	++++	+	0	0	0	0	0				
2 hr.	181	Tonsillitis	0	++	+++	+++	+++	+++	+	0	0	0	0	0	+	0	+	5.4
12 hr.	195	Tonsillitis	0	+	+++	+++	++++	++++	++++	+	0	0	0	0	+	0	+	5.4
12 hr.	120	Tonsillitis	0	+	++	++++	+++	++	+	0	0	0	0	0	+	0	+	5.35
12 hr.	129	Tonsillitis	0	0	0	+	+	++	+	+	0	0	0	0	+	0	+	5.40
12 hr.	177	Tonsillitis	0	0	+	+	+	+	+	+	+	0	0	0	+	0	+	5.50
12 hr.	200	Tonsillitis	0	0	+	+	++	++	+	+	0	0	0	0	+	0	+	5.15
12 hr.	201	Tonsillitis	0	0	+	+	+	+	0	0	0	0	0	0	+	0	+	5.50
12 hr.	198	Tonsillitis	0	0	+	+	+	+	+	+	0	0	0	0	+	0	+	5.50
12 hr.	Sc. 23	Scarlet Fever	0	++	+++	+++	++	++	+	0	0	0	0	0	+	0	+	5.25

+ —Slight flocculation without sedimentation.
++ —Moderate flocculation with slight sedimentation.
+++ —Marked flocculation with almost complete sedimentation.
++++ —Complete flocculation with clear supernatant.

It is obvious that acid agglutination has no value whatever in the differentiation of various strains of hemolytic streptococcus. On the contrary, the results indicate that these human strains of hemolytic streptococcus exhibit a fairly constant action in the presence of varying hydrogen-ion concentrations. A scarlet fever strain, demonstrated to be serologically distinct from other streptococci, in no way differs from them in respect to acid agglutination. This agrees with the work of Beniasch, though the agglutination optimum which he determined (pH 2.36) is considerably lower than that found by us (pH 3.6—3.9). The reason for this probably depends on the difference in the acid buffers used, in accordance with De Kruif's observations.

5. *Agglutination by immune sera.*—It was our purpose to classify the various strains from tonsillitis cases and carriers by specific agglutination and to relate the group to streptococci from other conditions such as scarlet fever. This work was not brought to a successful conclusion because of inability to obtain stable suspensions and potent immune sera.

A number of methods were tried in the hope of getting consistently stable suspensions:—Mass cultures were washed from plates of plain agar, blood agar and ascitic agar, and suspended in salt solution. Plain beef infusion broth of varying pH, ascitic broth, Huntoon's hormone broth, and broth containing a piece of raw potato, were tried with indifferent success, at both room and incubator temperature. An occasional strain grew diffusely on any of these media; the majority failed to do so on all of them. Some cultures which gave satisfactory suspensions would flocculate spontaneously at 56° in the water bath. Not infrequently, strains, growing diffusely for weeks, would over night become granular and remain so permanently, irrespective of subsequent manipulations.

So too, the greatest difficulty was encountered in the production of immune sera of high titer, tonsillitis strains being used as antigens. A multiplicity of tonsillitis strains having been demonstrated by other methods, further attempts at biological classification at this time were abandoned.

6. *Complement fixation.*—Swift and Kinsella,[11] and Kinsella,[12] have described complement fixation by streptococcus hemolyticus antigens in the presence of specific immune sera. Their strains came from a variety of sources but no differentiation was possible. All the organisms regardless of their origin showed cross-fixation with the various sera. It seemed of interest, however, to test complement fixation with the present series of strains. Twenty-four to forty-eight-hour hormone broth cultures killed at 65° C. for one hour were used as antigens. Immune sera of high agglutinative titer against a scarlet fever streptococcus and against a streptococcus from a case of impetigo (furnished by Dr. F. A. Stevens, and Dr. S. Bayne-Jones) were used. A rabbit anti-sheep hemolytic system was employed. The details of technique need not be set down except to say that adequate anti-complementary controls were always run both for antigen and immune serum and also a control to exclude hemolysis by hemotoxin. The results, which confirm those of Swift, are shown in Table III. No grouping of streptococci was possible by this method. Swift's series included only one scarlet fever strain. Inasmuch as the scarlatinal group has since been shown to be so distinctively agglutinated, it is of interest that our five scarlet strains fixed complement with the impetigo serum. The large number of negative tests with the scarlet fever antiserum are due to its low antibody content as indicated by titration against the hemologous strain (S. 23).

TABLE III.

Complement fixation by hemolytic streptococci

Strain No.	Scarlatina immune serum	Impetigo immune serum	Source of strain
203	0	+	Tonsillitis
119	+	+	Tonsillitis
1	+	+	Tonsillitis
218	0	+	Tonsillitis
117	0	+	Tonsillitis
195	0	+	Tonsillitis
166	0	+	Tonsillitis
180	0	+	Tonsillitis
120	0	+	Tonsillitis
118	0	+	Tonsillitis
129	0	+	Tonsillitis
199	0	+	Tonsillitis
S. 23	+	+	Scarlet Fever
S. B.	+	+	Scarlet Fever
S. A.	0	+	Scarlet Fever
S. C.	0	+	Scarlet Fever
S. F.	+	+	Scarlet Fever
D. A. L. 60	0	+	Dochez, Avery & Lancefield strain
D. A. L. 3	0	+	Dochez, Avery & Lancefield strain

In *summary*, then, for the present purpose it was found impracticable to employ all of the above methods, and our grouping of streptococci is based on (1) the appearance on the standard blood-agar plate, (2) fermentation of sugars, and (3) determination of the acid end-point. However, a combination of these methods yielded considerable information and seemed adequate to answer the main bacteriological epidemiological questions of the outbreak.

The following classification with the accompanying designations (Type A, Type B, etc.) has been adopted for briefness and convenience in the present report. Beyond that it has no value:

Fermentation of			Acid end-point	Designation
Salicin	Mannite	Lactose		
+	0	+	5.35+	Type A
+	0	+	5.20—5.05	Type B
+	0	+	4.9 —	Type C
+	+	+	5.35+	Type D
+	0	0	5.4 +	Type E
+	0	0	5.1 —	Type F
0	0	0	5.2	Type H

STRAINS OF HEMOLYTIC STREPTOCOCCUS FROM CASES OF ACUTE TONSILLITIS

From all of the 40 cases of acute tonsillitis hemolytic streptococci giving the *typical* beta appearance on blood-agar plates were isolated in pure or nearly pure culture. While strains from different people varied in the details of appearance both on the original plates and in subculture, all the colonies from a single case invariably seemed identical. This identity was borne out in the many instances in which two or more colonies from the same original culture were tested. Furthermore, frequent subcultures over a period of months did not lead to any change; the typical beta appearance was always preserved. Strains from 38 cases were classified by acid end-point and fermentation. The results are summarized in Table IV.

TABLE IV.

Grouping of strains of hemolytic streptococcus from thirty-eight cases of acute tonsillitis.

Type	No. of strains
A	28
B	2
D	6
E	2
	Total 38

It appears, then, that at least four varieties of beta hemolytic streptococcus were concerned with the present outbreak. Finer differential methods might have lead to even further subdivision. At any rate it is evident that no single epidemic strain was responsible for all of the cases. It is of interest, however, in contrast to the carrier strains, that 28 out of 38 of the present group fell into Type A. They thus have, on the one hand, the characteristics of the pyogenic streptococci (fermentation) and of the human type of streptococcus (acid end-point) on the other. It may also be mentioned that 19 of the 28 strains on duplicate observation gave an end-point of 5.40—5.45 and the widest limits of the group were 5.35 to 5.50.

STRAINS OF HEMOLYTIC STREPTOCOCCUS FROM CARRIERS

Fifty-six strains of hemolytic streptococci were isolated from the tonsils or pharynx of healthy carriers in the experimental group. The numbers varied from a few colonies (usually in cultures from the pharynx) to almost pure plates from tonsil crypt material. Forty-three of these strains originally presented and preserved on subculture the typical characteristics of the beta type. The remaining thirteen, while markedly hemolytic, showed slight deviations in so far as the zone of clearing was not absolutely complete, and slight degrees of greenish discoloration were evident on subculture. Forty-seven of these strains were studied by fermentation tests and the acid end-points were determined. The results are shown in Table V.

TABLE V.

Grouping of 43 strains of hemolytic streptococci from carriers.

Typical beta type		Atypical beta type	
Type	No. of strains	Type	No. of strains
A	21		
B	6	B	5
C	2	C	2
D	3		
F	2	F	1
H	1		
	Total 35		Total 8

Several points deserve comment:—

(1) Among the typical beta carrier strains type A predominated just as it did with the case strains. The relationship from the standpoint of epidemiology is obvious.

(2) Type B was found six times in carriers and was responsible for two cases. The topographical relationship of this group is of great interest, as will be shown later.

(3) Type D occurred in six cases and three carriers. Here also an epidemiological relationship was demonstrated.

(4) Type E which was responsible for two cases does not appear among the carrier strains which were grouped.

(5) Types C, F, and H, were recovered from carriers, but not from cases. There is reason to believe that the C strains were bovine types, although on the blood-agar plate they were indistinguishable from Type A (pyogenes). Their reactions together with those of known cow strains, kindly furnished by Dr. Jones of the Rockefeller Institute for Animal Research, are as follows:

Reactions of Type C strains and known bovine strains.

Number	Fermentation			Acid end-point
Type C—No. 27	+	0	+	4.70
Type C—No. 40	+	0	+	4.80
Type C—No. 89	+	0	+	4.70
Type C—No. 135	+	0	+	4.80
* Cow 53	+	0	+	4.75
* Cow M43	+	0	+	4.70

* Rockefeller Strain

It is also of interest that two of the four carriers of bovine strains had never had tonsillitis. As far as we know, the chronic focal tonsillar carriage of bovine streptococci has not hitherto been reported.

(6) The one strain of type H recovered from a carrier showed an unusual type of fermentation reaction. There was uncertainty as to how to classify it until we worked with a strain also obtained from Dr. Jones, labelled Cow 59, which showed identical fermentations and acid end-point.

(7) Finally, it is of note that none of the eight atypical beta strains gave the reactions of the group mainly concerned with tonsillitis (Type A).

Relationship of Carrier and Case Strains Derived from the Same Person

In a previous paper it was pointed out that people who were tonsil carriers of beta hemolytic streptococcus failed almost uniformly to develop tonsillitis, whereas the incidence of the disease in those who were not carriers was over 40 per cent. There were, however, four persons, previously carriers, who during the course of the experiment did develop acute tonsillitis and the question of the relation of the carrier and case strains from the same individual is of interest. The findings are shown in Table VI.

TABLE VI.

Comparison of carrier strains and case strains from the same person.

Case Number	Control culture		Culture during acute infection	
	Date	Strain	Date	Strain
190	Oct. 20, '22	Type B	Nov. 11, '22	Type A
89	Sep. 25, '22	Type C	Jan. 19, '23	Type A
40	Sep. 15, '22	Type C	Mar. 4, '23	Type A
12	Sep. 8, '22	Type A	Nov. 23, '22	Type A

In all four cases the tonsillitis was caused by Type A strains. Carriage of bovine streptococci, which as far as is known are non-pathogenic for man, in cases 89 and 40 failed to protect against subsequent acute infection. Carrier 190 later had tonsillitis caused by a new strain. Case 12 remains the only one in the series in which possibility of auto-infection with a carried strain can be considered.

In this connection an instance of tonsillitis followed by scarlet fever is of interest. Cultures from subject 156 on October 14, 1922, yielded no hemolytic streptococci. On January 4, 1923, she had an acute follicular tonsillitis, and cultures from pharynx and tonsils yielded typical beta hemolytic streptococcus in pure culture. Several colonies were tested and they all showed the characteristics of type B—fermentation of salicin, mannite, and lactose, and acid end-point 5.40. On February 17, 1923, the same person developed scarlet fever while nursing a scarlatinal patient. Throat culture again yielded beta streptococcus in pure culture but the scarlet strains all gave the reactions of type A—fermentation of lactose and salicin but not of mannite and acid end-point 5.40.

These observations are too few in number to allow conclusions, but they indicate at least that carriage of one variety of beta streptococcus does not necessarily protect against infection with other strains. Such findings, if they can be extended, will be of great importance in connection with the possible specificity of strains associated with various infections such as scarlet fever.

Persistence of Strains of Streptococci Following Tonsillitis

In the previous report statistical evidence was presented which indicated that carriage of beta streptococci in the tonsil usually dated from a preceding acute infection. It seemed probable that the duration of carriage might be quite long—perhaps a matter of years. Brown[2] on the other hand, working with surface throat swabs found that the real beta types were rapidly replaced during convalescence by alpha prime and alpha varieties. During the present work strains were isolated by crypt culture on successive occasions from the same person in several instances. Their reactions are summarized in Table VII.

TABLE VII.

Streptococci isolated from the same person on successive occasions.

Case Number	First culture			Second culture			
	Date	Reaction		Date	Reaction		Interval (days)
		L. M. S.	A. E. P.		L. M. S.	A. E. P.	
15	8/30/22	+++	5.35	1/30/23	+++	5.38	153
129	10/ 9/22	+0+	5.40	10/19/22	+0+	5.45	10
130	10/ 9/22	+0+	5.45	10/16/22	+0+	5.42	7
190	11/11/22	+0+	5.45	3/21/23	+0+	5.40	125

These few observations do not suggest any replacement during convalescence of case strains by beta streptococci of other types.

Discussion

An attempt has been made to classify strains of hemolytic streptococci obtained from cases of acute tonsillitis occurring in a limited group of people. The methods and their difficulties have been discussed. No wholly satisfactory technique for grouping streptococci has been developed as yet, nor can one say from a biological or pathological standpoint what the final logical division will turn out to be.

From the point of view of the immediate problem it has been possible to show that the cases of tonsillitis in the localized outbreak which we have studied were not all caused by a single strain of streptococcus, but that at least four varieties were concerned, and that a still greater variety were parasitic among carriers in the group.

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PRECIPITIN REACTIONS OF A CRYSTALLINE GLOBULIN FROM HUMAN URINE

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In connection with our recent studies on the immunological reactions of the Bence-Jones proteins [1,2] it occurred to us that immunological methods might be used to determine whether the crystalline globulin isolated from the urine of a patient by Noel Paton,[3] in 1892, was related to these substances. Professor Noel Paton had preserved specimens of this protein and kindly sent us two of them for examination. In addition to its possible relationship to the Bence-Jones proteins, the substance was especially interesting because of its property of crystallization. Through crystallization it could be obtained and used as a relatively pure protein antigen from man for comparison by appropriate tests with human serum proteins.

The history of the patient from whom the specimen was obtained was briefly as follows:

Dr. Byrom Bramwell saw the patient first in August, 1890. At that time, the patient, a white man, was forty-seven years old. He had always been in good health, and finally consulted a physician because of "lumbago" and hemorrhoids. The slight pain in the back disappeared in a few days. The patient's urine contained a large amount of protein, but no casts were found in the urinary sediment. There was no evidence of syphilis.

In September, 1890, the patient was somewhat thin and pale. Physical examination did not reveal any abnormality of the bones, muscles or internal organs. The only exceptional and outstanding abnormality was the large amount of protein in the urine, without any obvious evidence of nephritis. The patient excreted about 1400 c.c. of urine daily. The specific gravity of this was at times as high as 1.031. The protein content varied between 1.5 and 1.7 per cent.

The patient spent the next winter in Egypt, where he contracted a severe diarrhœa. He recovered from this in June, 1891, after his return to England. In January, 1892, he died of pneumonia following influenza.

Only an incomplete autopsy was made. No abnormalities of the bones were found, and there was no evidence of myeloma in the parts of the body examined. The kidneys were enlarged. Their capsules, however, could be readily stripped off, leaving a smooth surface. The kidney tissue was paler than normal, and was slightly scarred. In sections examined microscopically, no significant lesions of the renal epithelium were found. The liver was fatty and slightly cirrhotic.

No definite diagnosis could be made, either from the symptoms and examinations of the patient while he was alive, or from the post-mortem examination of his organs.

DESCRIPTION OF THE PROTEIN

When the urine of the patient was heated, coagulation of the protein began at 59°-60°C., and was complete at 62°C. In this process, at first a milky clouding appeared and then suddenly the coagulum changed to a fibrinous elastic mass. As the temperature was raised to the boiling point, the coagulum contracted, but did not dissolve. In remaining insoluble at 100°C. this protein differed from the true type of Bence-Jones protein.

The especially interesting property of this protein was its spontaneous crystallization in the urine. This occurred when the slightly acid urine was allowed to stand in a vessel for several days. The crystals were rather long white plates, somewhat similar in form to the crystals of oxyhemoglobin. In some preparations, the crystals were needle-like and occurred in clusters.

Noel Paton regarded the protein as a globulin. In 1896, Huppert [4] published a paper in which he expressed the belief that the substance was of the nature of a heteroalbumose. In 1898, however, after further study, Huppert [5] confirmed Noel Paton's opinion that the substance is a globulin.

We have received two specimens of this protein prepared by Noel Paton. They are designated in the experiments reported in this paper as N. P. 1. and N. P. 2.

Specimen N. P. 1: Protein precipitated by adding to the urine an equal volume of a saturated solution of ammonium sulphate. The precipitate was dialyzed, washed with water and dried. It is a tan-colored flaky powder, which dissolves in a slightly alkaline salt solution.

Specimen N. P. 2: Crystals of the protein from the urine, dialyzed, washed with distilled water, alcohol and ether, and dried over sulphuric acid. This is a finely granular white powder, which dissolves in an alkaline solution.

Immunological Reactions

These preparations of Noel Paton's protein were compared with human serum proteins and several Bence-Jones proteins by means of precipitin tests. The technique of these tests was the same as that described in previous papers.[1,2] The antisera for the reactions were prepared as follows:

Rabbit No. 106, was immunized to the non-crystalline specimen of protein, N. P. 1, by ten intravenous injections at intervals of three to four days. The first dose was 0.5 c.c. of a 1% solution, the last, 4 c.c. of a 2% solution. Eight days after the last injection, the animal was bled. At this time the serum of this rabbit gave a precipitate with a 1-3200 dilution of a 4% solution of the protein.

Rabbit No. 126, was immunized against the crystalline specimen of Noel Paton's protein, N. P. 2, by twelve intravenous injections at intervals of three to four days. The first dose was 0.5 c.c. of a 1% solution, the last, 5 c.c. of a 2% solution of the protein. Eight days after the last injection, when the serum gave a precipitate with 1-1600 dilution of a 4% solution of the protein, the animal was bled.

The other precipitating sera used were also obtained from rabbits; one from an animal immunized to human serum, the other, No. 144, immunized to the crystalline Bence-Jones protein No. 4.

The comparison of Noel Paton's protein with human serum proteins is shown in Tables I and II.

These precipitin reactions show that the crystalline preparation of Noel Paton's globlin is a different substance antigenically from human serum proteins. There is some crossing of the reactions of an anti-human serum with the salted-out preparation of Noel Paton's protein. This is undoubtedly due to impurities, traces of human serum proteins, in this specimen.

The comparison of Noel Paton's protein with Bence-Jones proteins was made by means of precipitin reactions with antisera No. 106 and No. 126 (antisera to the specimens of Noel Paton's protein) and five specimens of Bence-Jones protein. Of these, specimens Nos. 1 and 4 were the amorphous and crystalline preparations of Bence-Jones protein from H. M. R. which represented Group 1 of the Bence-Jones proteins. (2) Specimen No. 8, from patient J. E. D., represented Group 2. The results of these tests are shown in Tables III and IV.

In addition to the tests described above, precipitin reactions were made with the antiserum No. 144 from a rabbit immunized to the crystalline Bence-Jones protein No. 4, previously studied. This antiserum, after a year's storage in the ice-box, formed a precipitate with a dilution of 1-100,000 of its homologous Bence-Jones protein antigen, and gave a slight precipitate with other specimens of the Bence-Jones proteins. Solutions of both specimens of Noel Paton's protein were layered upon, and later mixed with, this antiserum. No trace of precipitate was formed.

These precipitin reactions demonstrate clearly that Noel Paton's protein has no antigenic similarity to any of a large group of Bence-Jones proteins which have been studied immunologically.

TABLE I.

Anti-human serum vs. Noel Paton's protein.

Anti-human serum Precipitation with antigen:	DILUTION OF ANTIGEN							
	0	1-10	1-100	1-200	1-400	1-800	1-1600	1-3200
N. P. 1 (1% sol.)	0	+	++	+	0	0	0	0
N. P. 2 (1% sol.)	0	0	0	0	0	0	0	0
Human serum	++++	++++	++++	+++	+++	++	++	+

TABLE II.

Anti-serum for Noel Paton's protein vs. human serum.

Serum	Precipitation with:	DILUTION OF ANTIGEN						
		0	1-100	1-200	1-400	1-800	1-1600	1-3200
106 anti- N. P. 1	N. P. 1 (4% sol.)	++++	+++	+++	++	++	+	+
	Human serum	0	0	0	0	0	0	0
126 anti- N. P. 2	N. P. 2 (4% sol.)	++++	+++	+++	++	+	+	0
	Human serum	0	0	0	0	0	0	0

TABLE III.

Precipitin reactions of antiserum to N. P. 1 vs. Bence-Jones proteins.

Serum 106 Anti-N. P. 1 Precipitation with antigens:	DILUTION OF ANTIGEN							
	0	1-10	1-100	1-200	1-400	1-800	1-1600	1-3200
N. P. 1 4%.....	++++	+++	+++	+++	++	++	+	+
N. P. 2 4%.....	++++	+++	+++	+++	++	++	+	+
H. M. R. 1 4%.....	0	0	0	0	0	0	0	0
H. M. R. 4 4%.....	0	0	0	0	0	0	0	0
J. E. D. 8 4%.....	0	0	0	0	0	0	0	0

TABLE IV.

Precipitin reactions of antiserum to N. P. 2 vs. Bence-Jones proteins.

Serum 126 Anti-N. P. 2 Precipitation with antigens:	DILUTION OF ANTIGEN						
	0	1-10	1-100	1-200	1-400	1-800	1-1600
N. P. 1 4%.....	++++	+++	+++	+++	++	+	+
N. P. 2 4%.....	++++	+++	+++	+++	++	+	+
H. M. R. 1 4%.....	0	0	0	0	0	0	0
H. M. R. 2 4%.....	0	0	0	0	0	0	0
J. E. D. 8 4%.....	0	0	0	0	0	0	0

CONCLUSIONS

1. The crystalline globulin isolated from human urine by Noel Paton is another example of a protein of human origin which can be sharply differentiated by the precipitin test from the proteins of human serum.

2. This crystalline protein differs from all specimens of Bence-Jones protein which we have studied, and possesses immunological properties peculiar to itself. On the basis of its immunological reactions and its failure to dissolve on boiling, we are of the opinion that it is not to be classified as a Bence-Jones protein.

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THE RATE OF LOCOMOTION OF AMEBA IN ALKALI CHLORIDES

By J. GRAHAM EDWARDS and HARRISON S. FORGRAVE, JR.

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In an investigation undertaken to discover the effect of chemicals on locomotion in Ameba, the senior author found that neutral salts in suitable concentrations in general permitted apparently normal locomotion for varying lengths of time. Such locomotion did not occur in any of the other chemicals used. It was evident that, even in neutral salts, the rate of locomotion varied with the salts used as well as with the concentration.

In order to ascertain first, the specific effect on locomotion of salts with different cations, four were selected with the same anion, namely, sodium, potassium, ammonium and lithium chloride. Chemically pure aqueous solutions were prepared of each of these in the following concentrations: N/100, N/300, N/500, N/700, N/900. For a given experiment four specimens of Ameba proteus were taken with a drop or two of culture fluid in a capillary pipette and immersed (1) in 25 c.c. of distilled water for 5 minutes, the dish being oscillated to facilitate diffusion; (2) in 25 c.c. for 3 minutes; (3) in 15 c.c. for 2 minutes. One of these amebas was then transferred to each of four Syracuse watch glasses containing 8 c.c. each of the salt solution to be tested. This was repeated until five amebas had been used for each concentration of each of the four salts.

The rate of locomotion was ascertained as follows: With the aid of a camera lucida and a Leitz monocular microscope the change in location of the posterior end was recorded on a sheet of paper every minute for 10 minutes. The advances were then measured and the actual rate in millimeters per minute calculated by making the

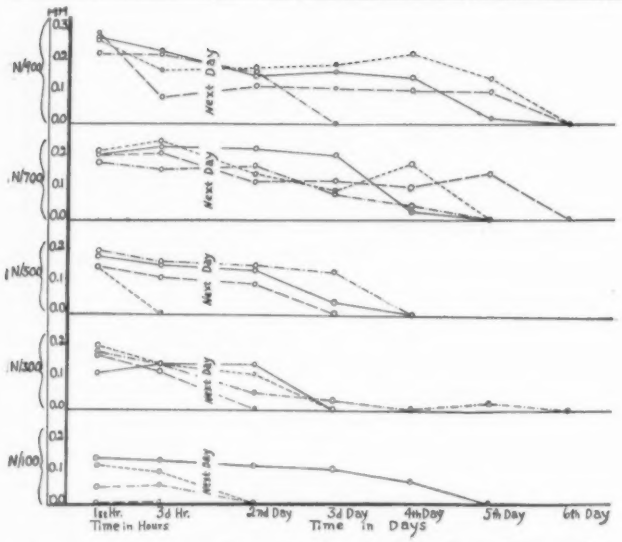

Fig. 1.—The rate of locomotion of ameba in sodium, potassium, ammonium and lithium chloride given in five graphs, one for each concentration showing the rate during five consecutive observations. Unbroken lines represent Na; long dash, —, K; short dash, -, NH_4; and a dash and a dot, —·, Li.

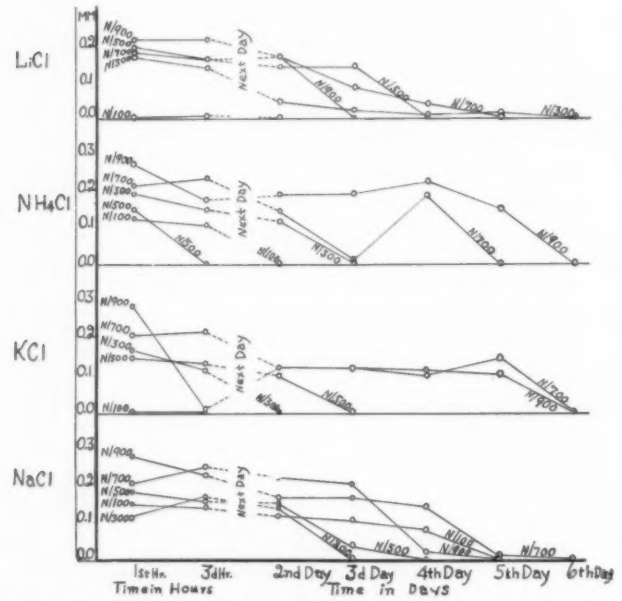

Fig. 2.—The rate of locomotion in each of the chlorides showing the difference in rate for each of the concentrations used for each salt.

proper allowance for magnification. Readings were made at the end of the first hour after immersion in a given concentration of each of the four salts, again at the end of the third hour and thereafter at the end of 24-hour intervals until locomotion became slight or inappreciable.

The results are presented in the accompanying table and in the graphs of figures 1, 2 and 3. These show the following:

1. The rate of locomotion in N/100 is greater for NaCl throughout the whole experiment than for any one of the other three salts (Fig. 1). Also, when all of the rates for the different periods and concentrations of sodium are added, it is found that more locomotion occurs in this than in any other of the chlorides and as follows: NaCl, 3.49518 cm., KCl, 2.27968 cm., NH_4Cl, 2.60607 cm., LiCl, 2.47677 cm. There is no locomotion in KCl N/100 at the end of the first hour.

2. The rate of locomotion for all of the salts in all concentrations tested decreases with the time the amebas are in the solution (Fig. 2). For example, the rate for NaCl N/100 at the end of the first hour was 0.1476 mm. per minute, while on the fourth day it was only 0.08 (see table). That the decrease in rate of locomotion is a function of the length of time during which amebas are in the solution, and not conditioned by some factor such as

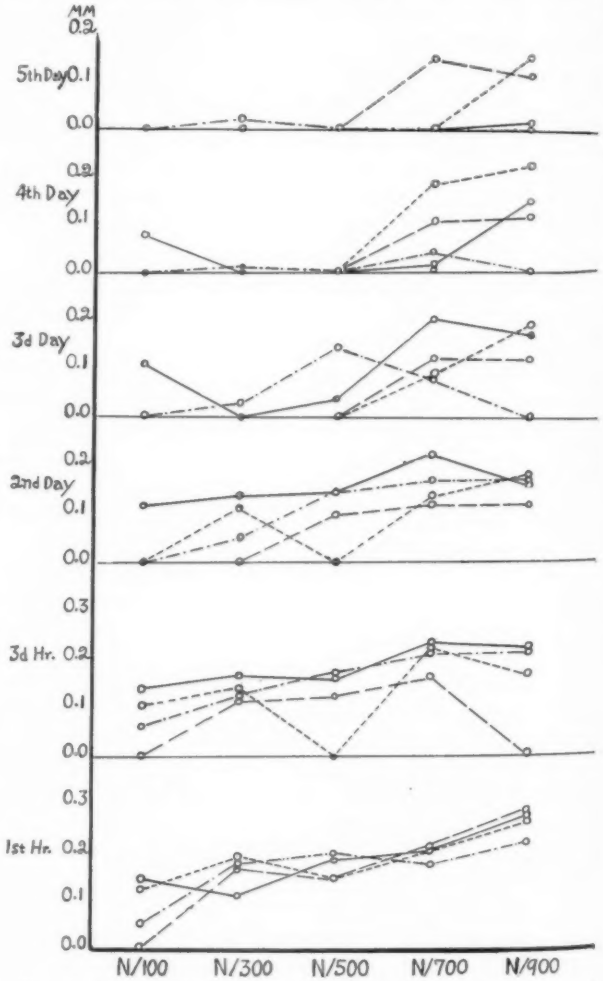

Fig. 3.—The rate of locomotion during each period of observation for each concentration of the four chlorides showing how markedly the rate increases with dilution and decreases with time. The different chlorides are indicated as in Fig. 1.

the absence of food, is indicated by the fact that the individual performance of amebas in a given concentration varies greatly. These variations are not shown in the averages used to obtain the rates given in the table. Therefore, the exceptions seen, for example, in N/700 Na, —K,—and NH_4Cl at the end of the third hour should not be considered as significant. If the absence of food were a factor, the rate would not increase with dilution and extend through so long a time (Fig. 3).

3. The rate of locomotion increases with a decrease in concentration (Fig. 3). In LiCl, for example, the rate at the end of the first hour was 0.05 in N/100 and 0.22 in N/900. The exceptions here may be regarded as in 2 above. The greatest rates at the end of the first hour are found in N/700 and N/900 for each of the chlorides used and, except in the case of lithium, amebas travel longer in these concentrations than in any other. The optimum concentration is N/900 (within the limits N/100 to N/900) for amebas in all the chlorides except lithium. This is true for the rate as well as the time during which locomotion is maintained (Fig. 3).

4. The rate of locomotion differs with the different salts (Fig. 1) and this is due to the cation. The cation, the time and the concentration are the significant factors affecting the rate of locomotion. With regard to other factors, such as osmotic pressure, temperature and surface tension, the first two need not be considered, since the osmotic pressure of the various solutions are so nearly the same that such slight differences as exist can be disregarded. Such osmotic pressures, determined by freezing-point lowering, are given in the tables compiled by Landolt, Börnstein and Roth. The temperature is the same for each series of experiments, therefore the influence of temperature may be disregarded. Surface tension effects cannot be discussed profitably, since there is at present no method for estimating the surface tension of protoplasm against salt solutions. Hence the conclusion seems permissible that the differences in rate of locomotion are conditioned by the specific action of the cations whether or not such action involves changes in the permeability or viscosity of the protoplasm or of surface tension or other possible factors.

Grateful acknowledgment is due Professor S. O. Mast for making the investigation possible, the experimental work having been done in his laboratory, and for helpful suggestions in preparing the manuscript.

Table showing average rate of locomotion in millimeters per minute of 5 amebas in various concentrations of sodium, potassium, ammonium and lithium chloride at the end of 1, 3, 24, 48, 72 and 96 hours. The rate given of the locomotion at the end of the first hour is taken as the initial rate for each salt and concentration. The figure in parenthesis following a given rate gives the number of amebas in actual locomotion at the time of observation.

Salt	Conc.	1st hour	3rd hour	2nd day	3rd day	4th day	5th day
NaCl	N/100	0.1476 (4)	0.1408 (4)	0.1220 (4)	0.1104 (1)	0.080 (1)	0.000 (0)
KCl	N/100	0.0000 (0)	0.0000 (0)	0.0000 (0)	0.0000 (0)	0.000 (0)	0.000 (0)
NH_4Cl	N/100	0.1280 (3)	0.1072 (2)	0.0000 (0)	0.0000 (0)	0.000 (0)	0.000 (0)
LiCl	N/100	0.05152(1)	0.0616 (1)	0.0000 (0)	0.0000 (0)	0.000 (0)	0.000 (0)
NaCl	N/300	0.1169 (4)	0.1691 (2)	0.1430 (2)	0.0000 (0)	0.000 (0)	0.000 (0)
KCl	N/300	0.1710 (4)	0.1160 (3)	0.0000 (0)	0.0000 (0)	0.000 (0)	0.000 (0)
NH_4Cl	N/300	0.19696(5)	0.1452 (4)	0.1168 (1)	0.0000 (0)	0.000 (0)	0.000 (0)
LiCl	N/300	0.17952(3)	0.14432(3)	0.0560 (1)	0.0297 (1)	0.00912(1)	0.0227 (1)
NaCl	N/500	0.18810(4)	0.1632 (4)	0.1490 (3)	0.0440 (1)	0.000 (0)	0.000 (0)
KCl	N/500	0.15168(5)	0.1267 (4)	0.1004 (3)	0.0000 (0)	0.000 (0)	0.000 (0)
NH_4Cl	N/500	0.15198(2)	0.0000 (0)	0.0000 (0)	0.0000 (0)	0.000 (0)	0.000 (0)
LiCl	N/500	0.20113(5)	0.16833(4)	0.1440 (0)	0.1472 (1)	0.000 (1)	0.000 (0)
NaCl	N/700	0.2095 (5)	0.2486 (5)	0.2269 (0)	0.20528(2)	0.022 (1)	0.000 (0)
KCl	N/700	0.2114 (4)	0.0587 (3)	0.1204 (3)	0.1232 (2)	0.104 (1)	0.149 (1)
NH_4Cl	N/700	0.21567(5)	0.230 (5)	0.1421 (3)	0.09168(2)	0.188 (1)	0.000 (0)
LiCl	N/700	0.18049(5)	0.1651 (2)	0.1720 (2)	0.08672(5)	0.040 (1)	0.000 (0)
NaCl	N/900	0.2825 (5)	0.2304 (4)	0.1678 (4)	0.17168(4)	0.1478 (2)	0.0056 (1)
KCl	N/900	0.2880 (4)	0.0935 (4)	0.1218 (2)	0.1218 (2)	0.1132 (2)	0.1089 (1)
NH_4Cl	N/900	0.27663(4)	0.1740 (3)	0.1844 (3)	0.18778(3)	0.22077(2)	0.1489 (1)
LiCl	N/900	0.22240(4)	0.2201 (2)	0.1739 (3)	0.0000 (0)	0.0000 (0)	0.000 (0)

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POIKILOCYTES IN OTHERWISE NORMAL BLOOD

(ELLIPTICAL HUMAN ERYTHROCYTES)

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Introduction

Of the two references to elliptical cells in human blood, the first is found in a report by Dresbach [1] of the Ohio State University, who in 1904 described the blood findings in a healthy mulatto male student, aged 22. Observations were made from time to time on his blood over a period of four months. The red corpuscles were subjected to the action of various reagents, and measurements were made of the size of the cells. The author states that such reagents as water, dilute caustic potash, dilute acetic acid, dilute hydrochloric acid, tannic acid, and others, were tried and that "the reaction was normal; but in each specimen taken many cells having the abnormal shape were noted. The erythrocytes were distinctly elliptical, slightly biconcave, non-nucleated cells, which did not adhere in rouleaux." * In many, biconcavity was scarcely perceptible, but 90% of the erythrocytes were estimated by the author not to have the circular outline of normal corpuscles. "These cells were elliptical whether subjected to coverglass or not." The author measured a large number of the corpuscles, and gave the extremes and averages of his measurements as follows:—width 3.9 to 4.8 μ, average 4.1 μ; length 8.5 to 10.7 μ, average 10.3 μ; ratio of width to length 1 : 2.5; average thickness 2 μ.

"The count was 5,000,000 per cu. mm. and the haemoglobin was up to standard. The white blood count presented no peculiarities."

Flint,[2] having seen the blood of this case and hearing that the man had died, became suspicious that the blood picture was not normal. In 1904 he published a note discussing the form changes in the erythrocytes and pointed out that, in progressive pernicious anaemia and other blood diseases, the variations in form and size are well known. He added: "In view of these facts, it seems impossible to accept the proposition that the subject of the observation was a healthy mulatto."

In answer to the preceding remarks of Flint, Dresbach [3] in 1905 replied:—"The cause of death was cardiac failure subsequent to an attack of acute inflammatory rheumatism. The attack was preceded by tonsillitis, which began about three months after the observations on the blood were made." And the writer states that consequently there was no connection between the condition of the corpuscles and the cause of death. He presented the following points to substantiate his claim that the patient was healthy. (1) His general physical condition indicated it. (2) He was able to make his living by manual labor, and was studying hard to maintain his standing in his classes. (3) He complained of no illness. (4) His haemoglobin, red blood count and white blood count were normal. (5) No normoblasts nor megaloblasts were ever found. (6) Flint says there was much poikilocytosis in his dry specimens. (7) Flint quotes from Ewing's "Pathology of the Blood" page 256 as follows:—"Sometimes in non-infectious purpura haemorrhagica the red corpuscles are undersized and many are oval." But Ewing himself saw the specimen of this blood and was of the opinion that the anomaly was one of congenital or developmental origin, basing his views, in the first place, upon the fact that the number of corpuscles and quantity of haemoglobin were normal, and in the second place upon the general condition of the individual himself. Ehrlich, Bleile, and Arneth saw the specimen of blood and thought that it was a congenital anomaly.

A second case report by Bishop [4] appeared in 1914. This patient was a white male, aged 21, who came to the surgical service of St. Luke's Hospital, N. Y. C. with the diagnosis of acute appendicitis. Examination of the stained smears revealed many elliptical red blood cells. Haemoglobin was 110% (Fleischl-Meischer) and the red blood count was 5,400,000. The patient gave a typical history of acute appendicitis. There were no lesions of the heart, kidneys or lungs. Examination of the abdomen revealed tenderness and rigidity of the right lower quadrant. Temperature 102°F. White blood count 16,500. Differential count revealed polymorphonuclears 82% and lymphocytes 18%. Urine was acid; specific gravity 1.015; albumin a faint trace; a few leucocytes were found microscopically. Operation was performed 2 hours after admission and there was found a gangrenous appendix with a small amount of seropurulent fluid free in the abdomen. Recovery was uneventful.

Careful study of the blood showed that 75 to 80% of the erythrocytes were elliptical in outline. Some were slightly elliptical, while others were very distinctly elongated. The following measurements were found:—circular cells measured 8.5 μ in diameter; length of the elongated cells 8.2 to 13.7 μ; width of the elongated cells 5.0 to 8.0 μ. Most of the elongated cells measured about 13.0 by 5.0 μ. Mechanical distortion of the cells was eliminated. Both fresh blood specimens and blood diluted

* It is not understood what the author means by this statement.

with Hayem's solution showed the anomaly. Fragility of the erythrocytes was normal. Vitally staining cells 3%. The blood Wassermann was negative.

The blood of the patient's sister showed the same condition. The haemoglobin was 100%, the red blood cell count 5,000,000, and 70 to 80% of the cells showed a more or less well marked elliptical outline. Few showed more extreme deformity than the patient's. One cell measured 17.2 by 3.2 μ and several were 15 μ long. The sister had never been ill. The father, the second sister and her two children, had normal cells. The mother of the patient was not living.

The patient was observed for 8 months. He remained healthy and always showed the same abnormality of the red cells. Haemoglobin determinations and red blood counts were always normal. The same is true of the blood of the sister who showed the condition. The author states; "As two members of the same family showed the same deformity, it is strong evidence in favor of the theory that the condition is a congenital anatomical defect."

The object of this present paper is to report another case which was observed for two years and to give a complete genealogical tree.

CASE REPORT

R.M.B. white, female, unmarried, age 28 years. Medical student.—She comes from an unusually healthy, long-lived family. All her immediate family are living and well. Two sisters have had operations for acute appendicitis, and there is also a history of acute appendicitis in a maternal uncle and aunt. There is a familial history of migraine on the maternal side, but none in the immediate family.

She has had the usual childhood diseases—whooping-cough, mumps and measles. Influenza and also a first attack of tonsillitis in 1922. She has had no other illnesses and her general health is very good. Menses began at the age of 12 years, are regular, intervals of 26 days with duration of 4 days.

Physical Examination.—Pulse 100, respirations 20, blood pressure 110/60. Temperature 98.6°F.

General color and nutrition good. Pupillary reactions normal. Tongue clear, and of good color. Throat not remarkable, thyroid normal. No general glandular enlargement. Lungs are clear everywhere. Heart normal in size and position, sounds clear. Pulse of normal rate and quality; peripheral vessels soft. Abdomen natural. Liver and spleen not felt; splenic dullness not increased. Extremities normal; no oedema. Reflexes active and equal. Height 5 feet 7¾ inches. Weight 156 pounds.

Laboratory Examinations.—The urine: specific gravity 1.020. No albumin or casts. 'Pthalein excretion: 70% of the dye excreted in two hours. Stool examinations revealed no ova or parasites. Wassermann reactions negative. Repeated tests showed normal fragility. Vitally staining cells varied between 1 and 2%. *Fresh preparations show that between 53 and 84%* of the erythrocytes have an elliptical or elongated form; hanging-drop preparation show from 59 to 70%; and stained smears from 12 to 40%. Haemoglobin determinations, red blood counts, white blood counts and platelet counts have been normal. (See Chart 1.)

The size of the red corpuscles varied little according to the method of preparation. The methods employed were as follows:—Fresh preparations were made by bringing a clean coverslip in contact with a drop of fresh blood from the finger-tip and mounting immediately on a glass slide and rimming with petroleum jelly. Hanging-drop preparations were made by placing a few drops of blood in an isotonic buffer solution and then transfering a drop of this suspension to a coverglass, which was then inverted and sealed with white oil on culture slides. Blood smears were made on coverglasses and stained with Wilson's stain and Ehrlich's stain.

The measurements of the cells prepared in the three ways mentioned above are shown in Table I.

TABLE I.

Preparations	Length μ		Breadth μ		Average of elongated cells μ	Circular cells measured μ	Percentage of cells having abnormal shapes
	Maximum	Minimum	Maximum	Minimum			
Fresh	16	4	7	2	12x5	6 to 11	53 to 84
Hanging drop..	16	6	7	3	12x5	4 to 8	59 to 70
Stained smears	16	7	6	2	10x4	5 to 9	12 to 40

The bloods of nine members of R. M. B.'s family were examined and none showed anything unusual except that of the patient's mother (Chart 2). Her blood revealed the same anomaly; from 50 to 80% of the erythrocytes in fresh preparations were of an elliptical or elongated form.

EXPERIMENTAL STUDIES

Experiments were carried out with the blood of R. M. B. and the following results were noted:—

1. Fresh preparations were made as described above. Immediate microscopical examination revealed elliptical or elongated forms varying between 53 and 84% in different preparations. Preparations observed every day over a period of three weeks or until they had deteriorated, showed no change in the percentage of abnormal forms (Fig. 1).

2. Hanging-drop preparations were made as described above. Immediate microscopical examination showed 59 to 70% of the cells to be elliptical or elongated. These preparations were observed for several days; there was no change in the percentage of abnormal forms.

3. The blood was placed in (a) isotonic buffer solution, (b) solution of 1.25% citrate in M/8 NaCl (isotonic salt solution), in physiological salt solution, (c) Hayem's

CHART I.

Date	Hb. %	R. B. C.	C. I.	W.B.C.	P.M.N. %	P.M.E. %	P.M.B. %	L. Lym. %	S. Lym. %	L.Mono. %	Trans. %	Myeloblasts %	Myelocytes N. %	Myelocytes E. %	Myelocytes %	Platelets
3/20/21	85	4,500,000	.9	6,000	65.6	3.0	.33	.66	21.3	2.33	6.66	0	0	0	0	260,000
10/10/22	90	5,500,000	.8	6,200	69.6	2.66	.33	1.0	22.6	.33	3.33	0	0	0	0	Estimated normal
3/27/23	90	5,544,000	.8	5,800	68.0	2.33	.66	.66	22.3	1.33	4.66	0	0	0	0	250,000

CHART II.

Name	Hb. %	R. B. C. millions	C. I.	Anisocytosis	Poikilocytosis	Basophilia	Nucleated Reds	W. B. C.	P. M. N. %	P. M. E. %	P. M. B. %	L. Lym. %	S. Lym. %	L. Mono. %	Trans. %	Myeloblasts %	N. Myelocytes %	E. Myelocytes %	B. Myelocytes %	Platelets
T. B.	85	4,904,.	.8	None	None	None	None	5,050	67.6	3.33	.33	0	17.0	3.0	8.66	0	0	0	0	Estimated Normal
B. B.	80	4,604,.	.8	Slight	Moderate	None	None	5,440	74.6	1.33	1.0	0	20.3	.66	2.0	0	0	0	0	Estimated Normal
R. B. H.	80	4,252,.	.8	None	None	None	None	5,240	73.6	2.33	0	0	16.6	3.66	3.66	0	0	0	0	Estimated Normal
R. B. W.	72	4,572,.	.8	None	None	None	None	7,320	70.6	1.33	1.0	0	20.3	1.0	5.66	0	0	0	0	Estimated Normal
R. V. B.	71	4,632,.	.77	None	None	None	None	7,100	71.3	1.66	0	0	16.3	2.0	8.66	0	0	0	0	Estimated Normal
L. H.	80	4,500,.	.8	None	None	None	None	6,000	64.6	2.33	0	0	25.6	2.0	5.33	0	0	0	0	Estimated Normal
S. H.	70	4,500,.	.7	None	None	None	None	6,000	60.6	1.33	0	0	27.0	3.33	7.66	0	0	0	0	Estimated Normal
E. H.	70	4,500,.	.7	None	None	None	None	5,500	37.0	3.0	0	3.0	49.3	3.0	5.33	0	0	0	0	Estimated Normal
E. H.	80	4,500,.	.8	None	None	None	None	6,000	39.0	1.0	0	1.66	48.3	3.66	6.33	0	0	0	0	Estimated Normal

solution, and a fifth series was set up in which the whole blood was oxalated. From these solutions fresh preparations were made and observed for three weeks. The cells were the same as in experiment 1.

4. Red blood cells from the patient were first washed in isotonic salt solution and then placed in the normal blood serum from a member of the same blood group (Group 2, Moss). Fresh preparations were made and sealed. These preparations were observed for three weeks. The cells revealed the same findings as in experiment 1.

5. Normal red blood cells of the same blood group as the patient's (Group 2, Moss) were washed three times in isotonic salt solution, and suspended in the patient's serum. Fresh preparations were made and sealed. The normal cells remained spherical over a period of three weeks, after which time the preparations dried.

6. The blood of the patient was placed in slightly hypotonic and slightly hypertonic salt solutions. From these fresh preparations were made and sealed. The elliptical and elongated forms occurred in the same percentages as in experiment 1.

7. The patient has been used several times as a donor in transfusions and no untowards effects have been noted in the recipients. In one patient with secondary anaemia, who recovered, no elliptical cells could be found after a period of two months.

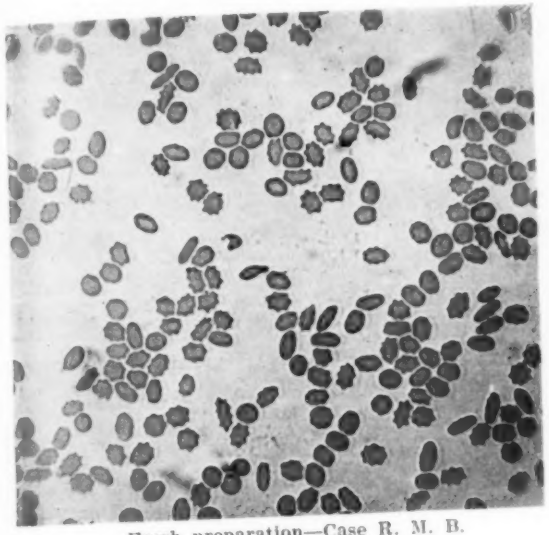
From these experiments it may be concluded that this particular or special poikilocytosis is an inherent property of the red blood cells or an anomaly of the bone marrow, and the patient's serum is without effect on normal erythrocytes. And that this anomaly cannot be transmitted by transfusion of blood.

Discussion

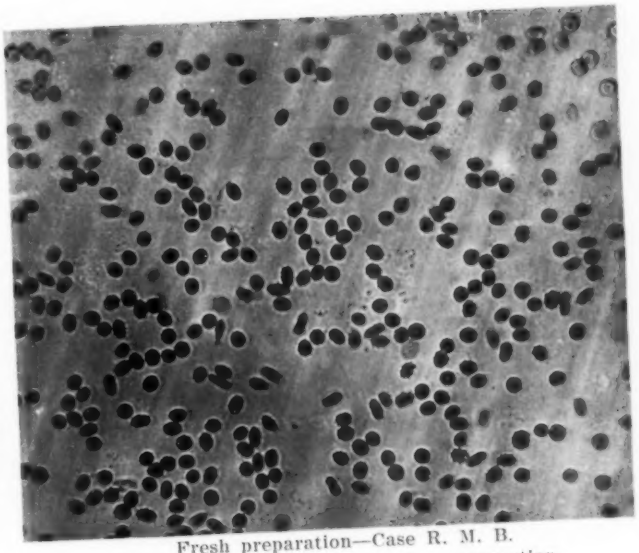
One may say that elliptical or elongated cells occur in otherwise healthy individuals, but very rarely. So far, only five cases have been observed, these occurring in the white as well as in black races. The ages of the subjects at the time when the condition recognized were between 21 and 51 years. Hereditary transmission, while not definitely established, in one case is strongly suggestive (Fig. 2). In this genealogical tree the female transmitted the condition. No other definite instance has been reported, although Bishop reported the blood findings of the father and two sisters of his case, only one sister showing the anomaly, and hence one may assume that here also the condition was transmitted by the mother. None of the other children from the parents of this case showed the condition. In this case the blood findings were normal except for the poikilocytosis. This condition is certainly not due to a mechanical phenomenon, inasmuch as the preparations were made by different methods and practically the same results were obtained from all. Other laboratory findings were all normal.

This anomaly of the red blood cells is inherent in these cells, and one cannot transmit the condition by intravenous injections of blood. The persons showing this condition may enjoy good health throughout life. The mother of our subject is still in good physical condition at the age of 51 years.

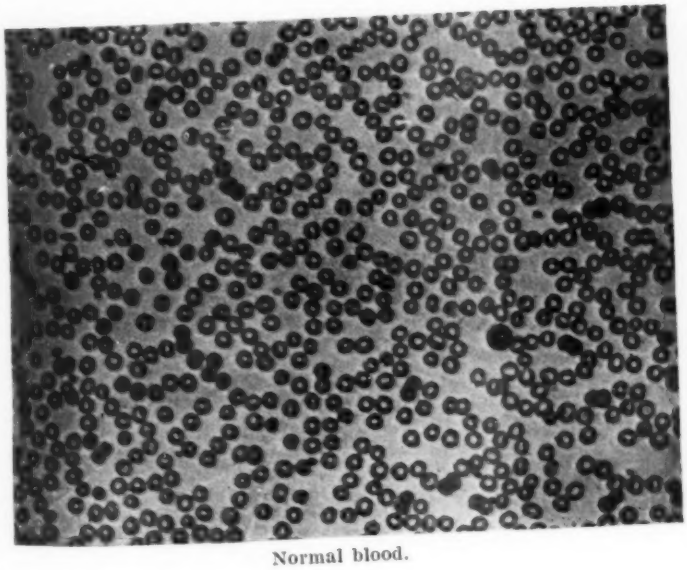
The pathology of these cases has not been described. The subject of Dresbach's report died from cardiac failure subsequent to an attack of acute inflammatory rheumatism, but no autopsy findings are at hand. The condition is in no way related to sickle-cell anaemia, despite the views of Cook and Myer[5] and V. Mason,[6] as one can readily see by comparison with the previous experimental studies by Huck[7] and the present work. In sickle-cell anaemia the red cells become abnormal in shape on standing, whereas the cells of the subjects described in the present paper are abnormal in shape immediately and remain so on standing, but do not change subsequently.



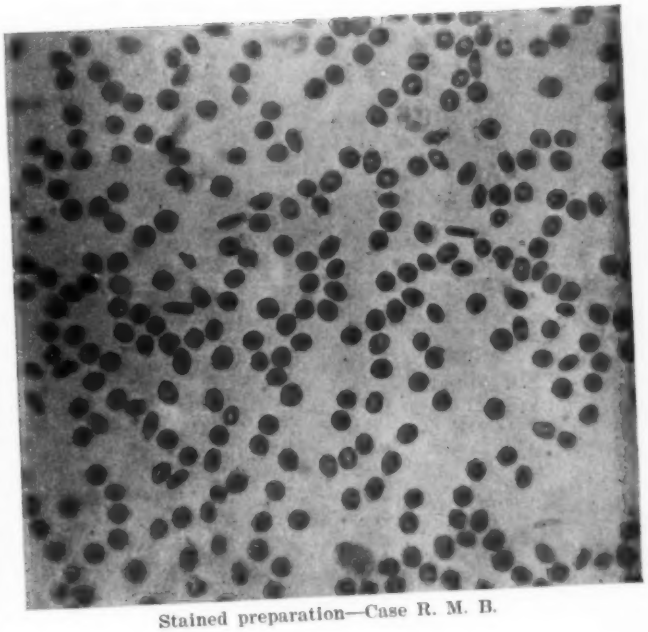
Fresh preparation—Case R. M. B.
Photographed immediately.



Fresh preparation—Case R. M. B.
Photographed three weeks after preparation.



Normal blood.



Stained preparation—Case R. M. B.

Fig. 1.

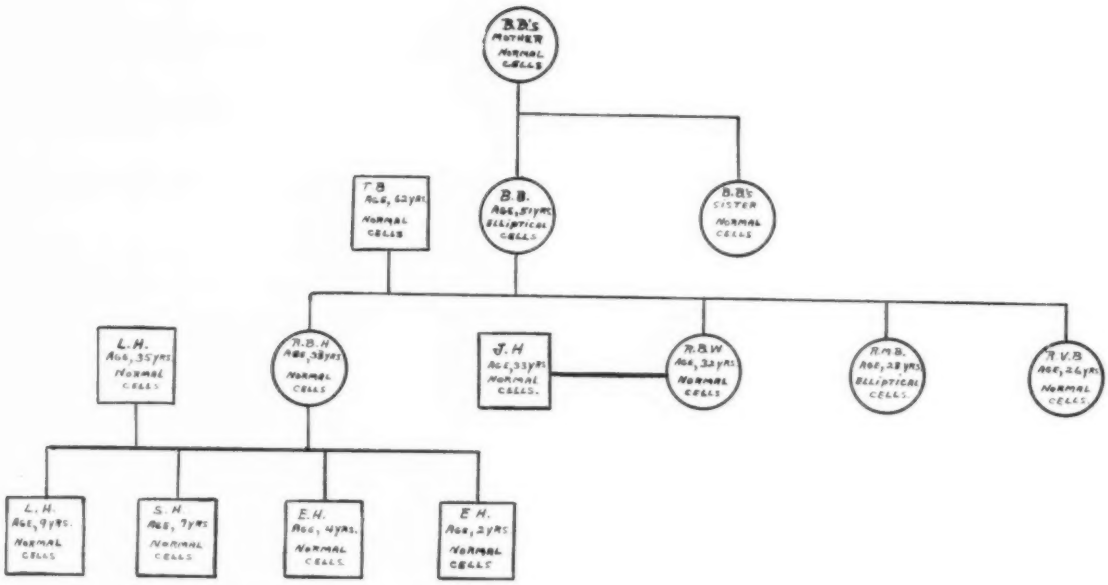

B.B's MOTHER NORMAL CELLS
T.B AGE, 62 yrs. NORMAL CELLS
B.B. AGE, 31 yrs. ELLIPTICAL CELLS
B.B's SISTER NORMAL CELLS
L.H. AGE, 35 yrs. NORMAL CELLS
R.B.H AGE, 38 yrs. NORMAL CELLS
J.H AGE, 33 yrs. NORMAL CELLS.
R.B.W AGE, 32 yrs. NORMAL CELLS
R.M.B. AGE, 28 yrs. ELLIPTICAL CELLS
R.V.B AGE, 26 yrs. NORMAL CELLS
L.H. AGE, 9 yrs. NORMAL CELLS
S.H AGE, 7 yrs. NORMAL CELLS
E.H. AGE, 4 yrs. NORMAL CELLS
E.H. AGE, 2 yrs. NORMAL CELLS

Fig. 2.

CONCLUSIONS

1. Poikilocytosis may occur in the blood of otherwise healthy persons of both the white and black races.

2. The condition is very rare.

3. The abnormal forms of the erythrocytes are most probably due to an anomaly of the blood-forming organs, and not to any outside influences.

4. So far as is known, the condition cannot be transmitted by transfusion of blood into other human beings.

5. Two definite findings differentiate this condition from sickle-cell anaemia: (a) no time interval is required for the red blood cells to change their shape, but they are abnormal in shape on immediate examination and remain so until the preparation deteriorates, whereas in sickle-cell anaemia the abnormal forms appear only after a time interval; (b) in sickle-cell anaemia, in which over 50% of the cells acquire bizarre forms on standing, there is an associated secondary anaemia, whereas in the condition described in this paper no anaemia is present even when 80% of the cells are abnormal.

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While the present report was in press, there appeared, in the Journal of the American Medical Association of July 14, 1923, a report of so-called "Elliptic Human Erythrocytes" by V. P. Sydenstricker. The patient was a negro man, aged 63, who had diabetes mellitus, epithelioma of the mouth, and syphilis, associated with a moderate grade of secondary anæmia, and a remarkable differential form. This author feels that his case is similar to those described by Dresbach and Bishop. If he had noted closely these authors' reports, he would have found that the conditions described were in healthy individuals whose blood was otherwise normal, and who had no associated disease. It is hardly likely that Sydenstricker's case is identical. It is a well recognized fact that anisocytosis and poikilocytosis occur in secondary anæmia. What Sydenstricker describes is probably a case of secondary anæmia due to syphilis, diabetes, arteriosclerosis, and advancing age.

ON THE MODE OF SPREAD OF AN OUTBREAK OF ACUTE TONSILLITIS*

By ARTHUR L. BLOOMFIELD and AUGUSTUS R. FELTY

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In previous reports [1] we have described an outbreak of acute tonsillitis which occurred during the six months from September 1, 1922, to April 1, 1923, among a group of people who were under constant clinical and bacteriological control—namely, the pupil nurses of the Johns Hopkins Hospital Training School.† Among a total of about 200 women there were some forty instances of the disease. It was pointed out that these cases did not represent a true epidemic in the sense of spread from person to person because a multiplicity of strains of streptococcus were concerned and because the temporal and spatial circumstances of the outbreak were such as to preclude, at least for the most part, direct infection. It seemed certain that healthy carriers scattered through the group were the sources from which disease arose. Furthermore, certain factors relative to susceptibility were defined;[2] it was shown that either tonsillectomy or carriage of beta hemolytic streptococcus protected against infection, and also that there was a group of people who seemed to possess a high degree of "natural" resistance. Of the susceptibles in the group—predicted on this basis —over two-thirds developed acute tonsillitis during the six months of the experiment.

It remained, then, to trace the details of the spread of streptococcus infection in the group and to relate the results to general principles of epidemiology. The present report concerns itself with this problem.

As pointed out above, the present outbreak of tonsillitis developed—at least in the main—not by case to case infection but by transfer from healthy carriers to susceptibles. The following possibilities, therefore, presented themselves in explanation of the mode of spread.

(1) Close contact—infection of room-mate by carrier.

(2) General contact—infection in the wards, assembly-rooms, dining room, etc., by general mingling of carriers and susceptibles.

Before discussing the data bearing on these possibilities it is necessary to describe the topography of the quarters in which the experimental group was housed.

The nurses' home (Diagram A) consists of three buildings. The first is divided into a central part (Main) and two wings (East and West). The second (B) is connected with the first by a corridor, and there are three floors (B1, B2, B3). The third building (318) is entirely outside the hospital grounds, about two city blocks away.

* This is the sixth of a series of papers on streptococcus infection with special reference to acute tonsillitis.

† The clinical and bacteriological features of tonsillitis have been discussed in detail in previous papers (Refer. 1-4) to which the reader is referred.

The students are disposed in these quarters according to classes as follows:

318—probationers
Main—juniors
B—intermediates
East & West—seniors

The seniors in the wings live in single rooms, in Main, and in B two students are in one room, and at 318 two or occasionally more probationers are quartered together.

All classes mingle (1) at daily assembly; (2) in the dining room, although the various classes have separate tables, (3) at work in the wards and operating rooms, (4) in the corridors and at the postoffice, and (5) at social gatherings (dances). There is, therefore, ample opportunity for contact among the members of the group as a whole.

An attempt was made first to trace the spread of the tonsillitis outbreak by a study of possible general contact. This was soon found to be hopeless because the assignments to duty and the other factors of movement were too variable, and the possible sources of infection (carriers) were too numerous.

A tabulation of the subjects of the experiment showed that there were twenty-five pairs of room-mates of whom one was a carrier and the other was free of beta streptococci. During the course of the winter seven, or 25 per cent, of these room-mates of carriers developed tonsillitis. When this figure was compared with the incidence in the entire group, which was 20 per cent, no significant difference was at first apparent. However, when the room-mates of carriers were classified from the standpoint of predictable susceptibility (tonsillectomy, previous history) it was found that only eight of the twenty-five were susceptible. Of these, seven, or 86 per cent, developed tonsillitis. These figures are summarized in Table I.

TABLE I.

Occurrence of tonsillitis in room-mates of carriers.

Carrier and non-carrier rooming together. Number of pairs	Number of cases of tonsillitis	Number of predictable susceptibles	Number of cases among susceptibles
28	7—25%	8	7=86%

It is seen, then, that close contact such as occurs between room-mates led to a very high rate of infection. However, only 7 of the total of 38 cases of tonsillitis which arose in the group could be accounted for in this way. A search was therefore made for other evidences of intimate contact which might explain the remaining 31 cases. When the number of occupants of the various dormitories, together with the case incidence, was tabulated, the following figures were obtained (Table II).

It appears that all of the cases occurred on floors where carriers resided. Beyond this crude fact nothing significant is brought out. However, when the location of carriers and cases is tabulated in connection with the type

TABLE II.

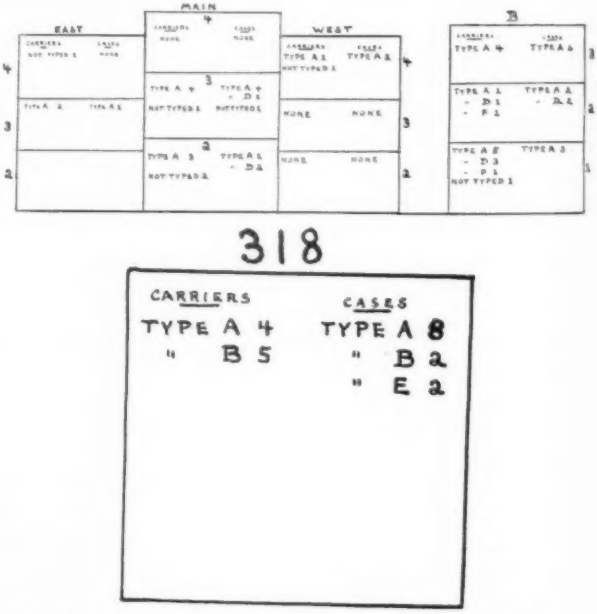
Incidence of carriers of beta streptococcus and of cases of tonsillitis in various dormitories.

Dormitory	Number of occupants	Number of carriers	Number of cases
318	37	9	12
B 1	25	10	3
B 2	14	3	4
B 3	25	4	6
Main 2	24	5	3
Main 3	20	5	6
Main 4	3	0	0
East 3	7	2	1
East 4	10	1	0
West 3	6	0	0
West 4	7	2	2

of strain concerned, certain obvious epidemiological relationships appear. In the preceding paper[3] the classification of the hemolytic streptococci isolated from carriers and from cases of tonsillitis was discussed in detail and it was pointed out that in the present work eight different varieties were distinguishable. They were designated for convenience Type A, B, C, etc. In Diagram A the topographical distribution of these strains in the members of the experimental group is presented. The following points were of interest.

DIAGRAM A.

Location of cases and carriers according to strains



Type B.—In the entire series there were five carriers of Type B strains and two cases of Type B infection (tonsillitis). It is immediately evident from the diagram that the whole group was confined to 318. In other words, although the occupants of 318 were in general contact with the remainder of the nurses as pointed out above, all the carriers of Type B and the only two cases

of the entire outbreak caused by Type B were found in this unit.

Type E.—No carriers of this strain were discovered. The two cases both occurred in occupants of 318, and developed at about the same time—January 6th and January 12th—although the patients were not room-mates.

Type D.—A consideration of Type D shows that all the carriers were originally concentrated on B1 and B2. Two cases of Type D infection arose on B2. This grouping is quite inexplicable on a basis of general contact and must, we think, be due to special local intimacy. A second group of 3 cases of Type D infection occurred in Main 2 and Main 3. No relation of any sort could be traced between this group and the B group of D infections. There were three carriers in Main 2 and 3, who unfortunately were not grouped and who may have harbored Type D strains. However, the three cases in Main 2 and 3 occurred on January 4th (1), and on January 5th (2), so that here again an intimate relationship seems warranted.

Type A.—The Type A carriers and cases were so numerous that no focal relationship could be traced, but the significant fact may be noted that no Type A cases occurred in a unit in which Type A carriers were not also present.

In summary, then, the topographical data on Type B, Type E, and Type D cases seemed to prove that infection was essentially a matter of dormitory contact and was not the result of general mingling of carriers and susceptibles in the wards, assembly rooms, etc. In the absence of evidence to the contrary, it appeared likely that the same was true of the Type A infections.

If this hypothesis was correct, it seemed that clinical information should be forthcoming which would be corroborative. The following statement was furnished by the supervisor of the nurses' home: "The occupants of each unit belong to the same class. There is practically no intimate social contact between nurses outside of their own class-mates. Every room seems to belong to all the other dwellers on the floor. They constantly gather in the rooms when off duty, as many as six to ten at once, or as many as can squeeze in, and remain together laughing and talking and eating for hours at a time." Under these circumstances the rooms also become hot and humid—factors which promote droplet infection.

It seems fair to conclude then that occupants of the same floor or of the same unit were in essentially as close contact as room-mates and that all or practically all of the cases of tonsillitis were dormitory infections.

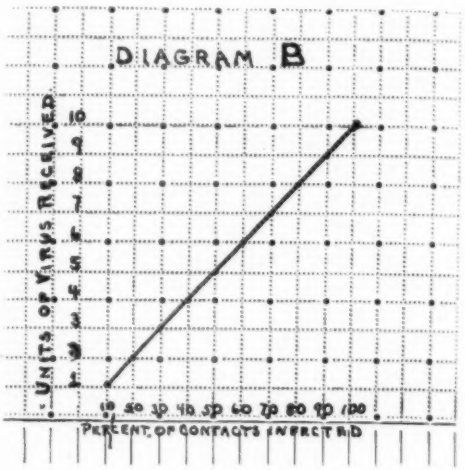
In this connection a theoretical question of great importance in epidemiology is brought up. There was obviously more opportunity for infection in the dormitories than elsewhere, but none the less, considerable contact must have occurred throughout the group. It is remarkable that no evidence at all was forthcoming of infection resulting from general mingling of the nurses in the dining hall, assembly, and wards.

The matter seems explicable however in terms of a principle formulated by Dudley [5] and spoken of by him as the "velocity of infection." This subject seems of such essential importance that we quote Dudley in considerable detail. Dudley's observations were made in connection with an outbreak of diphtheria in a large boys' school. He writes as follows:

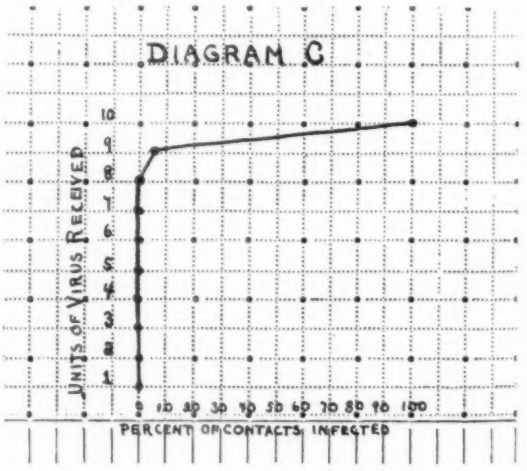
"*Velocity of Infection.*—For reasons given in previous sections it is almost certain that far more boy-to-boy infection took place in the dormitories than outside them, though at meals and in class boys were much nearer together than in beds with 4½ feet between their centres, in which occupants were arranged head to foot. The above rather peculiar observation can be explained by a principle which seems very obvious to the writer but which he has never seen clearly enunciated elsewhere. If there is anything in this theory it may be of considerable help in solving some problems of preventive medicine. In many, or all, infectious diseases there is a certain minimal dose of infective agent necessary to cause the disease. If a dose of the infective agent smaller than this 'minimum infective dose' (M.I.D.) is received by an individual, it is destroyed by the defensive mechanisms of the body. The M.I.D., of course, varies with every host and every parasite, but probably remains between certain limits for the majority of ordinary susceptibles. The above statements are in accordance with many clinical observations and laboratory experiments. If a subject receives a number of sub-minimal doses, which, when totalled, exceed a M.I.D., it is reasonable to suppose that it must depend on the rapidity with which these fractional doses are received as to whether or not he falls a victim to the disease in question, that is to say, as to whether the rate at which the doses of infective agent can be dealt with is greater than the rate at which they are received. The study of the spread of disease in ships, or elsewhere, has forced the writer to the conclusion that in many bacterial infections it is more often the rule than not for an infective dose to be collected by degrees rather than received as a whole in one moment of time. Perhaps the use of algebraical symbols will make the meaning of this introduction of the time factor into the question of bacterial infection clearer. For the sake of argument, suppose that all the conditions are fixed and constant. An individual receives V amount of infective agent per hour. The defensive mechanism can destroy U amount of this infective agent per hour; then we may term V the velocity at which infection is received and U the velocity of destruction of infection. If U is greater than V, it follows that no infection can ever take place so long as the conditions remain unchanged, because the infective agent is being dealt with as fast as it can be received. But if V is greater than U, infection can take place, but only if the conditions are in operation long enough. The time required will depend on how much greater V is than U, that is, on $V-U$, which is the amount of the infective agent left undestroyed at the end of each hour, and therefore $V-U$ is the velocity at which infection takes place. The nearer U (the velocity of destruction) approaches V (the velocity of reception), the smaller $V-U$ (the velocity of infection) becomes. When U becomes greater than V the velocity of infection is a negative quantity, and the numerical size of this quantity is an index of the degree of immunity *under the special conditions existing* at the time. V will of course vary with the source of infection and its distance, and with the conditions of ventilation and humidity. It is now easy to see that for a subject to collect a M.I.D.

of an infective agent he must remain $\frac{\text{M.I.D.}}{V-U}$ hours in the same infective environment. Applying this principle to this school we can see that if $\frac{\text{M.I.D.}}{V-U}$ was generally more than one but less than ten hours, the absence of case-to-case infection in the class-rooms and at meals, and the heavy incidence of the same in the dormitories, is easily explained. The complete absence of morbidity among the day-boys was the point that was so puzzling; a low morbidity in comparison with that of the residents would have been easily accounted for, as due to less time spent in the school and therefore less opportunity of getting a single infective dose; but it needs the principle of velocity of infection to explain how over 100 boys played, worked and ate for over a year without returning a single case of diphtheria or scarlet fever, while 300 of the boys with whom they played, worked, and ate developed one or other of these diseases. This theory of velocity of infection seems to offer a reasonable explanation of some well-known facts that are difficult to understand without it. It has been experimentally ascertained that infected 'droplets' of saliva will carry as far as 30 or 40 feet. However, it is also a fact that a ward full of coughing children can be treated in beds 9 to 12 feet apart without cross infections occurring. Now it is surely more easy to believe that cross infections are so rare because infective material is being received at such a low rate and in such low concentration at 12 feet, as never to exceed the rate it can be dealt with (i. e., $V-U$ is always negative), than to suppose that infective material never gets as far as the 9 to 12 feet which are the fundamental principle of bed isolation treatment. It will be noted that nine feet is only double the distance between the bed centres in the dormitories of this school. It does not seem at first that this difference in distance would be great enough to account for the safety of bed isolation compared with the danger of boy-to-boy infection in the school dormitories. It must be remembered that the likelihood of infection falls off very rapidly with distance. If the linear dimensions of any space are doubled, the volume is increased eight times and the concentration of any infection in the original space becomes correspondingly reduced; thus at 9 feet the concentration would only be one-eighth of what it was at 4½ feet, and at 12 feet the concentration only one-nineteenth; consequently, on theoretical grounds, the concentration of infection decreases directly as the cube of the distance from the source of infection. Direct experiment seems to confirm this. Glover increased the distances between the centre of soldiers' beds from about 3 to 5 feet, and roughly, 3 cubed is to 5 cubed as 1:5. The carrier rate for meningococci dropped from 28 to 6 per cent (roughly 4.7: 1), which is practically the cube of the inverse ratio of the two distances between bed centres. Therefore, on these considerations, by doubling the distance between bed centres the velocity of infection would drop from $V-U$ to $\frac{V}{8}-U$."

Dudley's principle, in a word, makes it necessary to distinguish between the mere physical bringing together of source and susceptible which *might* result in transfer of an infective dose of virus and which may be spoken of as *potential contact*, and the actual completed transference from person to person of an infective dose which may be spoken of as *adequate contact*. The need of distinguishing the two is brought out if we make a graphic chart to illustrate the significance of Dudley's formula. If the effectiveness of contact, *ceteris paribus*, varied directly with the dose of virus received per unit of time by the susceptible person, then the chance of infection should vary directly with the dose; or if one unit of virus per unit of time is adequate to infect in 10 per cent of the cases, then 5 units should be effective in 50 per cent of the cases and 10 units in 100 per cent of the cases (see Diagram B).



This straightline curve fits in with the conventional ideas of infection. According to Dudley's formula, however, if 10 units equals the minimum dose which, *ceteris paribus*, is adequate to produce infection in 100% of susceptible contacts, then 9 units will infect not 90 per cent but a much smaller number—approximately zero. (See Diagram C.)



In terms of Diagram C the chances for infection in the present outbreak of tonsillitis are immediately explained; if diagram B is correct, the situation as found is inexplicable.

More in detail, it is clear that general resistance to tonsillitis in the group was great. This is shown by the fact that such simple factors as carriage of streptococci,

prophylactic inoculation, and tonsillectomy were highly effective in preventing the disease, and that even when contact was prolonged and intimate, as in the case of room-mates, weeks and sometimes months elapsed before infection finally came about. It is obvious, then, that an intensive transference of virus was necessary in the present instance to produce infection. If it be assumed hypothetically that the amount of virus received by a susceptible from a heavily infected carrier during continuous conversation for one hour at close range in a small room constitutes a minimum infecting dose, and if this value be substituted in Dudley's formula, it immediately becomes apparent that such contact conditions as might occur through general mingling of the group would be less likely to be adequate in the ratio indicated in Diagram C. In a word, the only opportunity for adequate contact probably came about when the nurses were crowded into small closed rooms, engaged in active talking, laughing and coughing.

A further application of the above principle served to explain an observation which had previously been totally obscure, namely, the seasonal distribution of tonsillitis in the group. A study of the incidence of tonsillitis in students of the training school for the past three years showed that the disease was practically absent in the months of May, June, July, August and September. In October the cases began to appear, increasing slightly in number until March. Toward the end of March and in April there was a rapid falling off. The curves are presented in detail in another paper.[4] In order to explain this seasonal variation an intensive study was made to discover some factor which varied with the time of year. The following points received consideration:

1. *Susceptibility.*—As pointed out above, it has been possible very definitely to define susceptibility to tonsillitis.[2] Carriers of beta-hemolytic streptococcus are practically immune, non-carriers are highly susceptible. Tonsillectomy confers almost complete protection. Finally, there are a certain number of people who have never had tonsillitis at all and who seem to be "naturally" immune. In the face of these concrete factors, it seems unwarranted to assume some additional general subtle seasonal fluctuation in resistance of the whole group which results in infection of 75% of susceptibles in winter and in absence of infection in summer.

2. *Virulence of the virus.*—The assumption of seasonal alterations in the virulence of streptococci in chronic carriers would be wholly gratuitous. The number of carriers remains the same summer and winter and no experimental alteration of virulence has been demonstrable.[6]

3. *Activity of susceptibles.*—The nursing routine continues unaltered throughout the twelve months of the year.

4. *Weather, etc.*—An intensive study, reported elsewhere,[4] of the present outbreak of tonsillitis together with the weather, temperature, sunshine, humidity, etc., gave no hint of any relationship.

By exclusion, then, attention was again directed to the factor of concentration in the dormitories. Further information from the supervisor of nurses was to the following effect. "As soon as spring weather appears foregathering in the dormitory rooms practically stops. The nurses when off duty go out of the hospital or occupy themselves on the large hospital lawns. They go into their rooms practically only to go to bed. Outdoor life ceases at about the first of October when the days get short and the nights are cold."

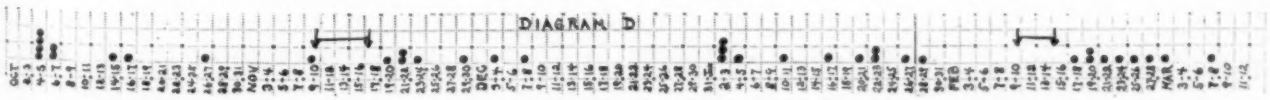
It is clear then that the only demonstrable variable in the situation is the type of crowding which has been shown to be adequate for infection and this varies almost mathematically with the occurrence of tonsillitis.

One more point remains to be explained. Diagram D shows the temporal occurrence of the cases of tonsillitis during the course of the winter.

It is noted that from October 18th to November 21st, from December 9th to January 3rd, and from February 1st to February 20th, there are relatively long breaks during which practically no cases occurred. This hardly seems accidental. A possible explanation was suggested by periodic rearrangement of rooms as new groups of probationers enter the school. An investigation disclosed the fact indicated on the diagram by arrows that from November 10th to 17th there was a rearrangement of quarters involving some sixty nurses, and from February 10th to 15th another equally large rearrangement. It is possible that from time to time states of equilibrium were reached as most of the susceptibles had come down and that the regrouping led to renewed possibilities for intimate contact with resulting increase of case incidence.

Discussion

An epidemiological study of tonsillitis has been made with the idea that accurate bacteriological, clinical, and topographical data when combined might be more significant than mere statistics on incidence. By correlating previously determined factors of susceptibility, the variety of streptococcus, and the spatial distribution of cases it has been possible to show that the present outbreak represented a series of infections of susceptibles by carriers and that intensive crowding was necessary to bring about transfer of virus of a degree adequate to produce disease. In the present instance such crowding was found to occur in the dormitories during hours of recreation. It was also shown that the striking seasonal variation in the occurrence of tonsillitis could be explained by cessation of crowding during the summer months.



In brief all of the phenomena of this outbreak have been explained in terms of definable factors. It has been of interest to us that this result was brought about without the need of falling back on subtle, mysterious, or inexplicable influences. This seems to indicate the inadequacy of incomplete data if epidemiological phenomena are to be defined.

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NOTES ON NEW BOOKS

Dietetics for Nurses. By FAIRFAX T. PROUDFIT. *(New York, Macmillan & Co., 1921.)*

Dietetics for Nurses by Proudfit is sound in its theory and written in an interesting manner. The menus and calculated recipes for the preparation of food for the sick make it a practical handbook for every nurse. The discussion of diet in diabetes mellitus is obselete, but in every other respect the dietary regulations for various diseases are complete and up to date in every particular. Personally, I find the book very valuable as a text-book and also for reference.

L. O. C.

The Anatomy and Physiology of the Capillaries. By AUGUST KROGH, Ph. D., LL. D. *(New Haven, Yale University Press, 1922.)*

Though it needs no argument to prove that the capillaries constitute the functioning zone of the entire vascular system and that the heart and arteries exist but to supply the capillaries with blood, nevertheless the study of the physiology of the capillaries has lagged far behind the analysis of the function of the rest of the vascular system. As Krogh points out, during the past seven years an increased interest in the capillaries has been "in the air;" indeed his own brilliant studies with those of his students, brought together in this book, make the best sort of a scientific contribution, being a record of much accomplished and of stimulus which will carry the subject much farther in the coming years.

Krogh has taken the fact, familiar to all with experience in injecting muscles, that results are exceedingly varied in regard to the number of capillaries which are filled in an injection, and has illuminated those differences in terms of varying physiological states at the time of injection. Almost everyone else has discarded the incomplete injections as failures; Krogh has analyzed them. He injected India ink into the blood-stream of an anaesthetized animal and then killed the animal while the muscles were resting, moderately stimulated or tetanized. In the last experiment the muscles were black from the number of capillaries filled, that is, showed a complete injection; in the other two, varying numbers of capillaries were filled, but it was very striking that in both conditions the open capillaries were uniformly distributed throughout the muscle. This fact gives the analysis in terms of function. In watching the capillaries at the base of the nail in the human skin, Miss Carrier, one of his pupils, has observed an orderly sequence in the closing of the capillaries. A similar closing of the capillaries of entire glomeruli in the kidney has been observed by Richards and Wearn. Krogh then analyzes two types of organs, one like the muscles, in which, in the resting state, but few capillaries are open while during function many or all of them are open; the other, like the skin, where the usual condition is an average of comparatively low metabolism when the capillaries open one after another in an orderly sequence. Perhaps a third type could be found in a gland like the pancreas, where for a long period of functional activity the color of the tissue remains the same and the cells histologically show a uniform amount of the granules that are the forerunners of secretion throughout the organ at any one moment. In these studies Krogh makes a plea for extensive measurements of the capillary surfaces involved, in order to make a basis for the study of the varying rates of oxygen-exchange.

The work of a group of modern physiologists, Krogh and his students, Dale and Richards, Hooker, and Rich have revived the earlier work of Stricker and demonstrated completely that there is an independent contractility of capillaries. Krogh believes, on the basis of the work of Vimtrup, one of his pupils, that this contractility is not the property of the endothelial cell itself but of certain branched cells of the connective tissues, scattered along the capillaries, the so-called Rouget cells. I cannot but think that the refined modern histological technique for studying living capillaries in the tadpole's tail, used by Vimtrup and by Clark and Clark in this country, can settle this problem, for it is now possible to watch the capillaries with an oil-immersion lens for hours or days, though it must be admitted that contractility is one of the most difficult of vital phenomena to analyze by direct observation. Nevertheless, with vital dyes it should be possible to tell whether the endothelial cell or the Rouget cell initiates contractility and whether one or both are active.

Besides this very illuminating point of the types of variation in the capillaries as regulating different types of function, Krogh has made a great advance in the subject of the mechanism for the regulation of the capillaries. Hooker demonstrated that there is a control of the capillaries by the sympathetic nervous system. Krogh finds that there is a double nervous mechanism. The capillaries are contracted by means of the sympathetic nerves from the thoracic cord and dilated by means of "antidromic fibers" whose cells lie in the dorsal ganglia and whose fibers follow the sensory nerves. Krogh also gives evidence to indicate that the facts demonstrable from local stimulation of capillaries can be completely analyzed only on the hypothesis of the existence of peripheral nerve nets. In this connection it is interesting to recall that in the studies in which Harrison demonstrated so conclusively the outgrowth of the nerve fiber from a single nerve cell in amphibia he also showed nerve nets, while Lewis and Lewis subsequently cultivated very extensive nerve nets from the sympathetic nerve cells of the intestine of the chick. Besides the double control by the nervous system, there is also a tonic contraction of the capillaries due to a hormone. The effects of histamine on the capillaries has been demonstrated by Dale and Richards, Hooker and Rich, as well as by Krogh and his pupils. Krogh showed first, by studying the capillaries after all nervous control has been eliminated, that there is an effect due to a hormone. He then demonstrated that in the frog this hormone can be eliminated by the removal of the hypophysis, and that the posterior lobe and pars intermedia produce this hormone. He then demonstrated that there

is a hormone in mammalian blood, which he obtained by dialyzing the blood of the ox, which has the same effects on the capillaries and melanophores of the amphibian as their own pituitary extract. By a crucial experiment he demonstrated that the hormone controlling the capillaries is identical with the pituitary hormone by perfusing one leg of a frog with Ringer and the other with Ringer plus the dialysate obtained from the ox-blood, the latter maintaining the power over the capillaries and over the pigment cells as well.

On page 65 Krogh suggests the possibility of direct connections between arteries and veins as a normal occurrence. In one of the chicks which I was growing on a cover-slip I had an opportunity to watch an arterio-venous fistula. It was between the omphalo-mesenteric artery and the accompanying vein close to the origin of the artery from the aorta. When I first opened the chick which was of the 3rd day of incubation there was a small persistent connection between the two vessels, the vein arising from branches of the artery which usually separate entirely from the parent vessels. In less than an hour this fistula had enlarged so much that, instead of an occasional corpuscle passing directly from artery to vein, most of the current of the blood was swept through it like a vortex and so was deflected from the normal capillary bed. I am convinced that had development continued the embryo must have become abnormal. The observation makes me skeptical about a persistent direct connection between an artery and a vein being compatible with the normal development.

In the final chapter Krogh deals especially with a phase of the subject which indeed is suggested throughout the book, namely, with the bearing of the study of the capillaries on general problems. He suggests the study of the process of absorption in three especial places, in the thorax under the condition of negative pressure, from the alimentary canal and from the aqueous humor into the canal of Schlemm. He then shows that beside the study of normal metabolism a complete analysis of the function of the capillaries is a necessary foundation for the study of the cause of oedema. Many interesting observations have been omitted in this review, but it is hoped that enough of the value of the book has been indicated to show that no student of the structure and function of the vascular system can afford to miss a careful study of the work of Krogh.

F. R. S.

Tonsillectomy by Means of the Alveolar Eminence of the Mandible and a Guillotine. By GREENFIELD SLUDER, M. D. *(St. Louis, C. V. Mosby Co., 1923.)*

The object of this monograph is to present the author's method of tonsillectomy. The successive stages of the operation are described in detail and the text is supplemented by excellent illustrations by J. M. Heller. The last 74 pages are devoted to operative technic together with pre-operative and post-operative care, anaesthesia and post-operative complications. The author considers nitrous oxide the anaesthetic of choice and operates with the patient in the recumbent position. He claims that in the hands of one trained in the "Sluder Technic" a perfect tonsillectomy can be performed in 99.6% of all cases. He reports a series of 20,000 tonsillectomies under nitrous oxide in the service of the Laryngological Department of the Washington University School of Medicine with no post-operative cases of sepsis or lung abscess. There was one case of pneumonia which he ascribes to the abolition of the cough reflex by morphine. Little is said of post-operative hemorrhage.

The first 88 pages of the book are devoted to a general review of the embryology, physiology and pathology of the tonsil and to the indications for and the contraindications against tonsillectomy.

The chapter on physiology and general pathology has been written by Arthur W. Proetz and includes a discussion of the five theories of tonsil function. The pathology of the author's series of cases is not discussed. It would be interesting to know the percentage of tuberculosis in 20,000 cases.

The last chapter is by I. D. Kelly, Jr. and deals with adenoidectomy under direct vision. He describes and illustrates an instrument developed for this purpose. The work contains 90 illustrations and is supplemented by a bibliography of some 142 references, some of which are incorrectly quoted in the text.

J. J. C.

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